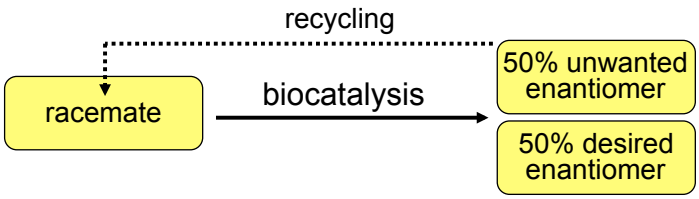
Engineering Enzymes for Non-Natural Carboligation Reactions

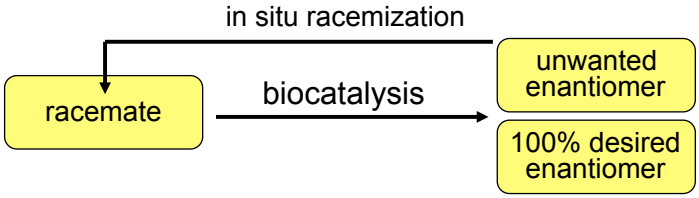
Wolf-Dieter Fessner
TU Darmstadt
Germany

Routes to Chiral Building Blocks

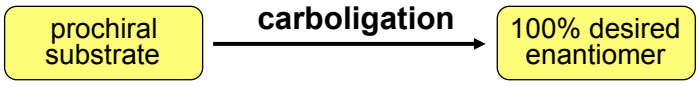
kinetic resolution
several steps
50% yield limit



dynamic resolution
several enzymes
100% yield



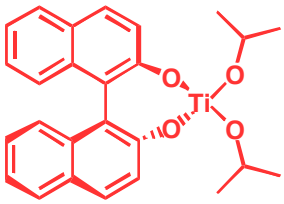
asymmetric synthesis
one enzyme, one step
100% yield



Catalytic Asymmetric Carboligation

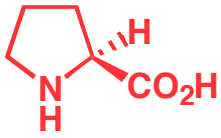
Multiple Opportunities

Metal Catalysis → chiral Lewis catalysts



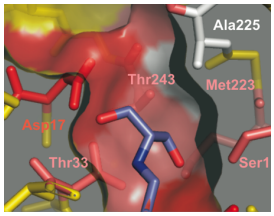
Productivity

Organocatalysis → small molecule organic catalysts



Versatility

Biocatalysis → **aldolases** (abzymes)

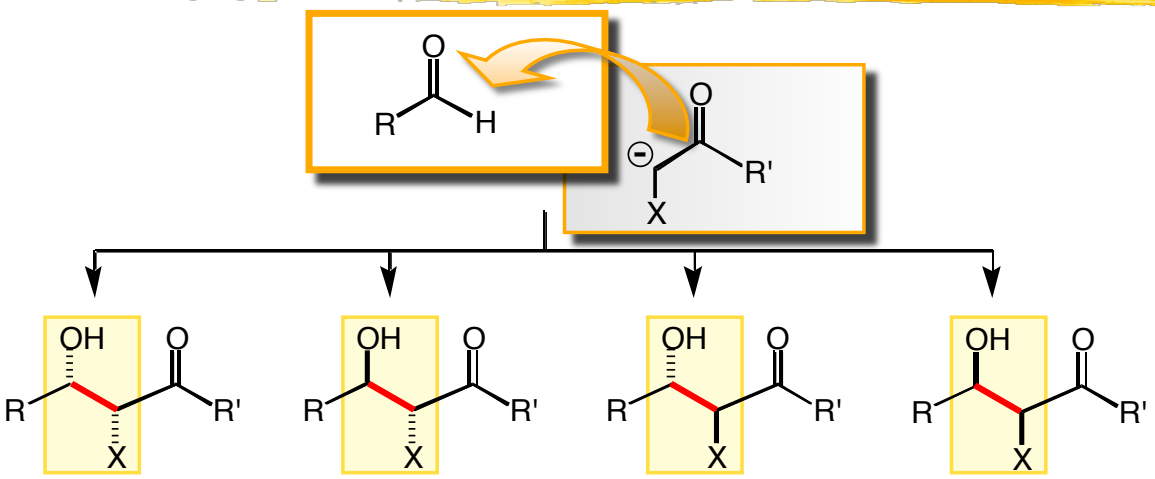


Selectivity

Overview

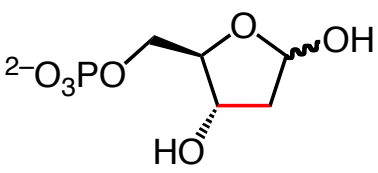
- **Asymmetric Carboligation**
  - Fructose-6-phosphate Aldolase from *E. coli*
  - Transketolase from *G. stearothermophilus*

Aldol Opportunity

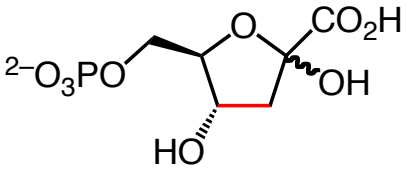


high stereoselectivity, fully controlled by enzyme
broad substrate tolerance for acceptor component
many enzymes well studied & commercially available

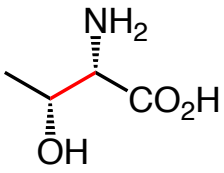
Nature‘s Aldol Products



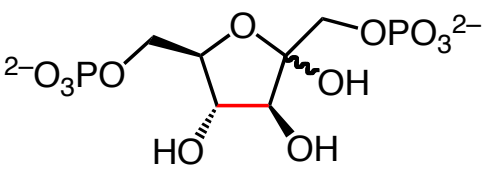
2-deoxy-D-ribose 5-phosphate



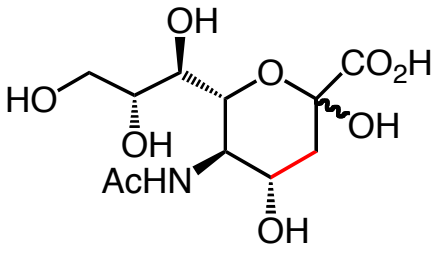
2-keto-3-deoxy-6-phospho-D-gluconate



L-threonine

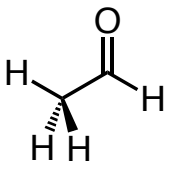


D-fructose 1,6-bisphosphate

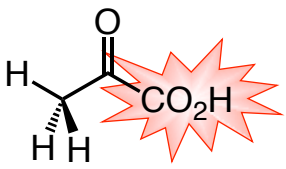


N-acetyl-D-neuraminic acid

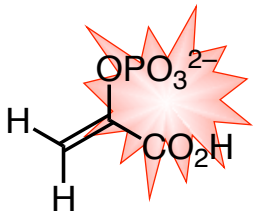
Nature‘s Nucleophiles



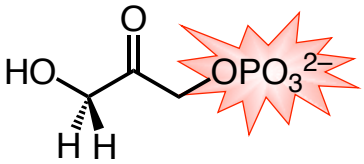
acetaldehyde



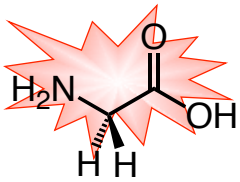
pyruvate



phosphoenol-pyruvate



dihydroxyacetone phosphate



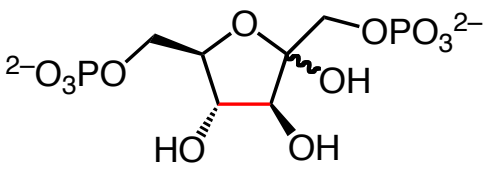
glycine

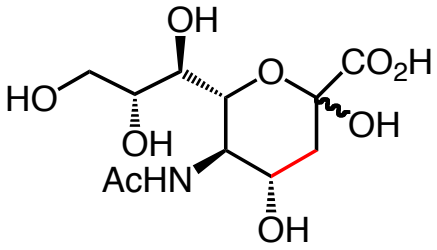
all are prochiral

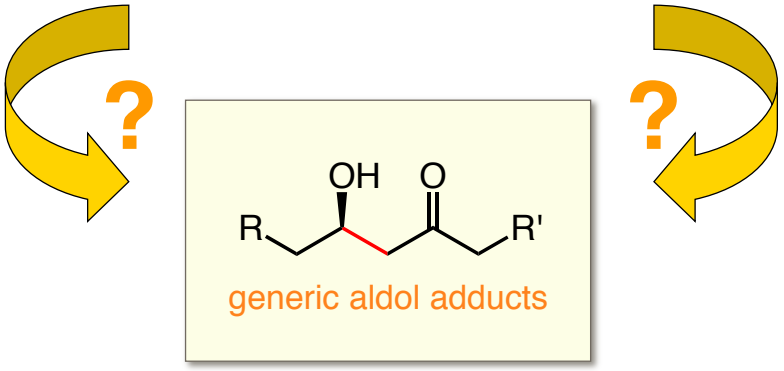
most are highly polar

Fessner et al. *Curr. Opin. Chem. Biol.*, **2010**, *14*, 154-167

The Challenge





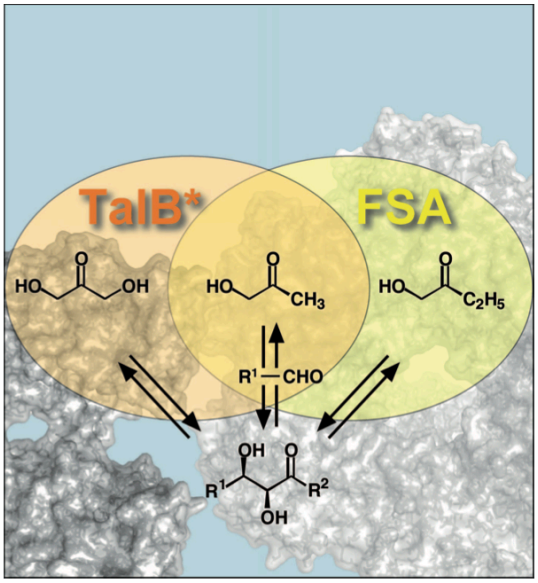


New Enzymes: FSA + TalB^{F178Y}

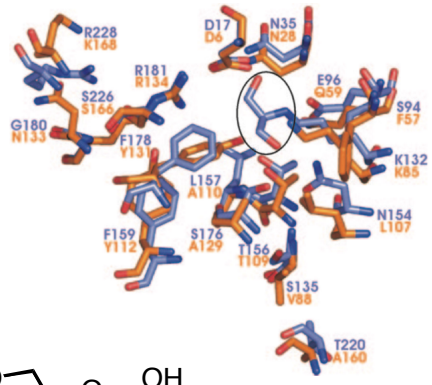
CHEMBIOCHEM
DOI: 10.1002/cbic.201100072

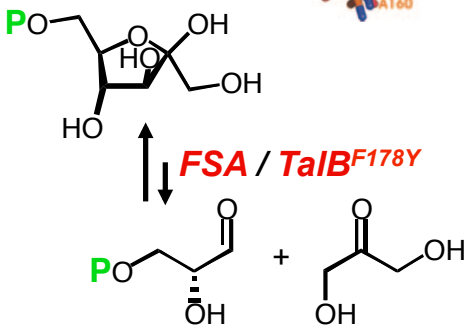
The Transaldolase Family: New Synthetic Opportunities from an Ancient Enzyme Scaffold

Anne K. Samland,[a] Madhura Rale,[b] Georg A. Sprenger,[a] and Wolf-Dieter Fessner*[b]

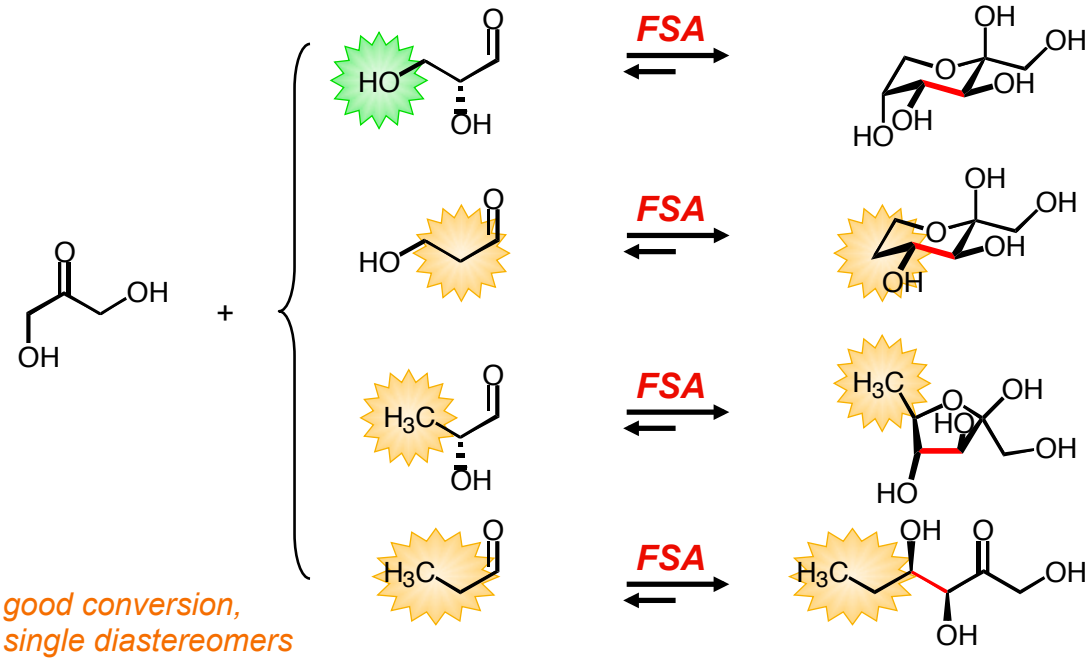


1454 © 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim ChemBioChem 2011, 12, 1454 – 1474





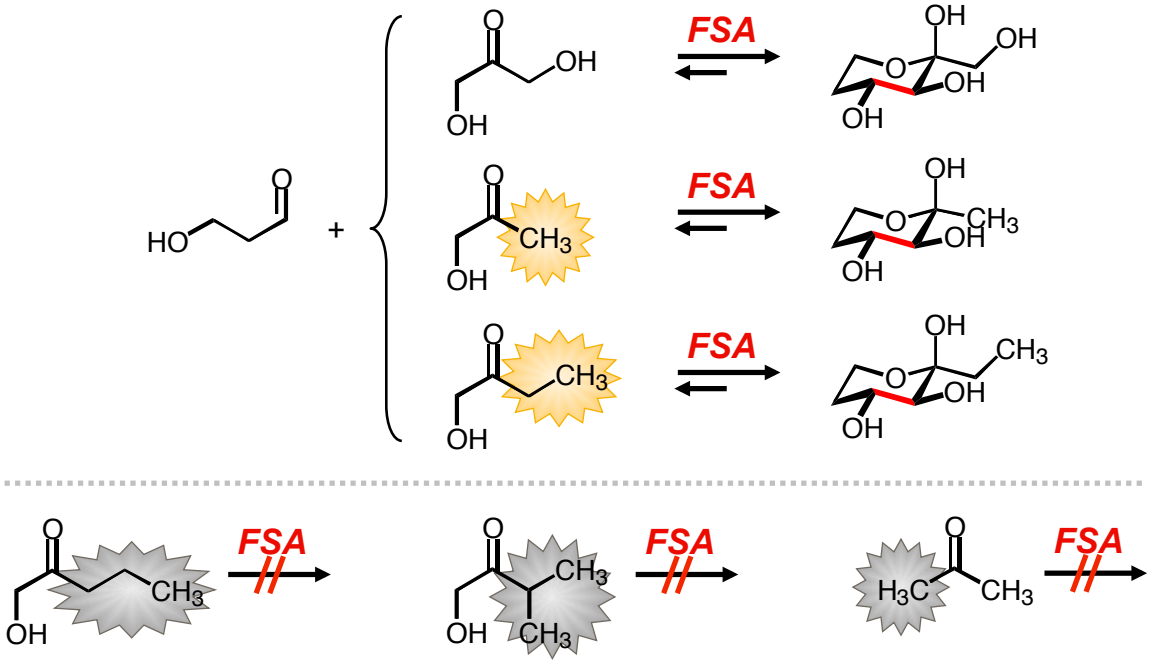
Substrate Tolerance: Electrophiles



good conversion, single diastereomers

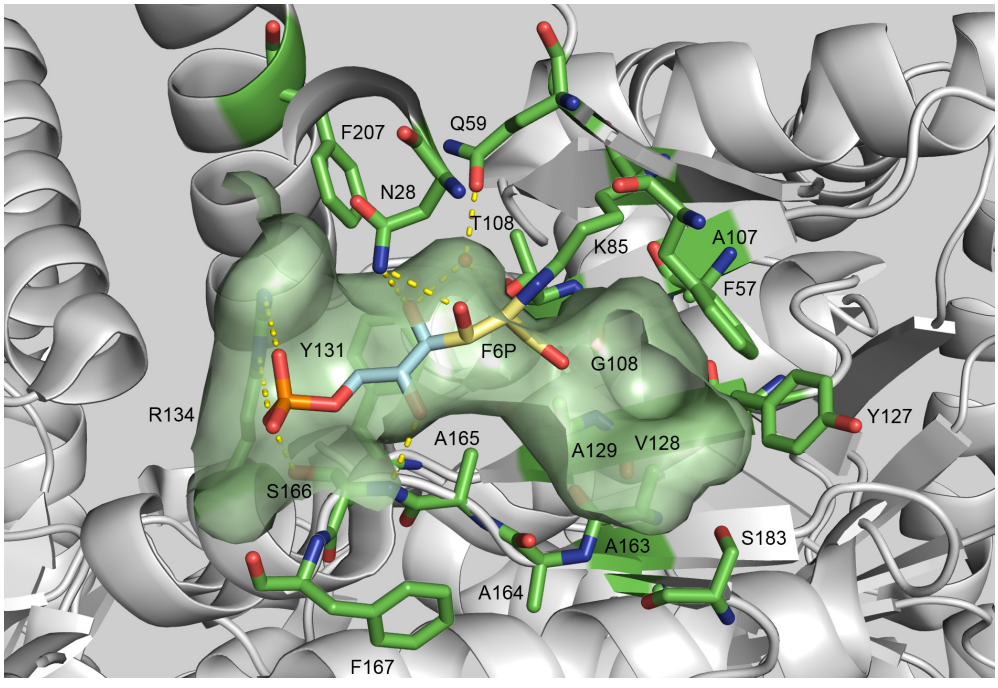
Fessner et al. *Chem. Eur. J.*, **2011**, *17*, 2623-2632

Substrate Tolerance: Nucleophiles



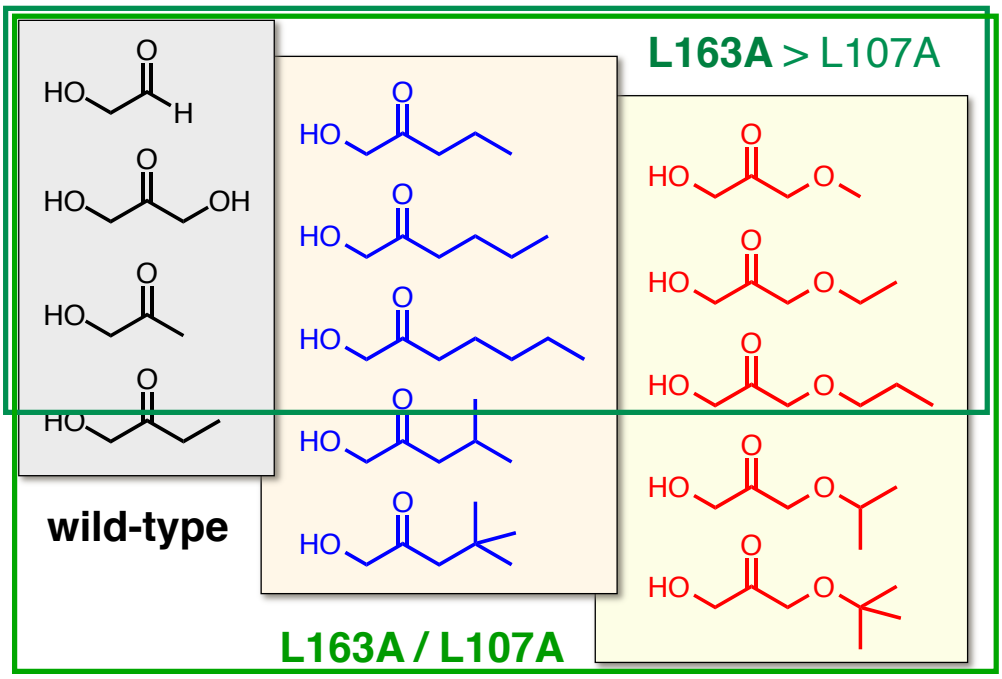
Fessner et al. *Chem. Eur. J.*, **2011**, *17*, 2623-2632

Protein Engineering



structure-based active-site mutations to expand donor tolerance

Donor Variations



Fessner et al. *manuscript in preparation*

Protein Stability

Destabilization by Leu→Ala Cavity-Creating Mutations:

Matthews et al. *Science* **1992**, *255*, 178-183

$$\Delta\Delta G = 2.0 \text{ kcal mol}^{-1} + n \times (0.03 \text{ kcal mol}^{-1})$$

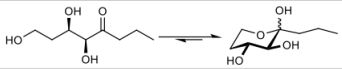
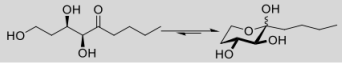
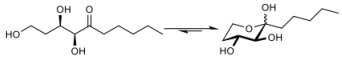
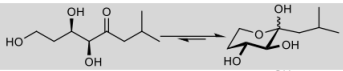
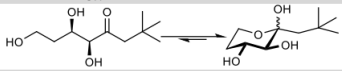
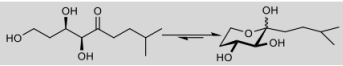
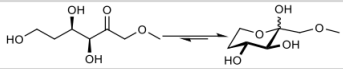
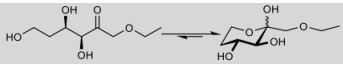
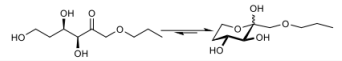
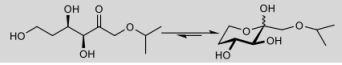
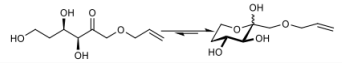
transfer constant *cavity volume term per Å³*

Variant	T_m [°C]
FSA_{wt}	87.0
FSA (L107A)	90.4
FSA (L163A)	78.4
FSA (L107A / L163A)	81.4

Differential Scanning Fluorimetry

Pantoliano et al. *J. Biomol. Screening* **2001**, *6*, 429-440

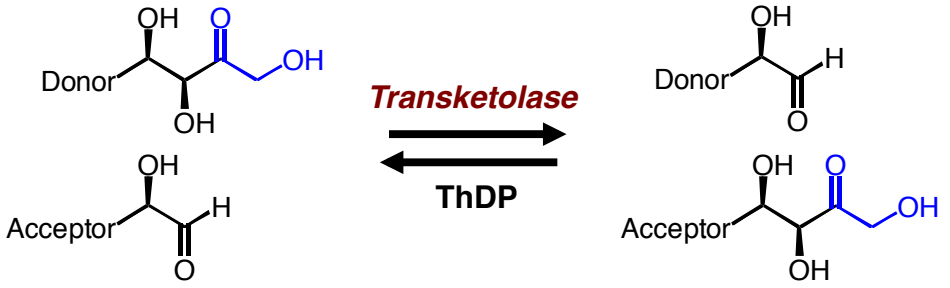
Product Range

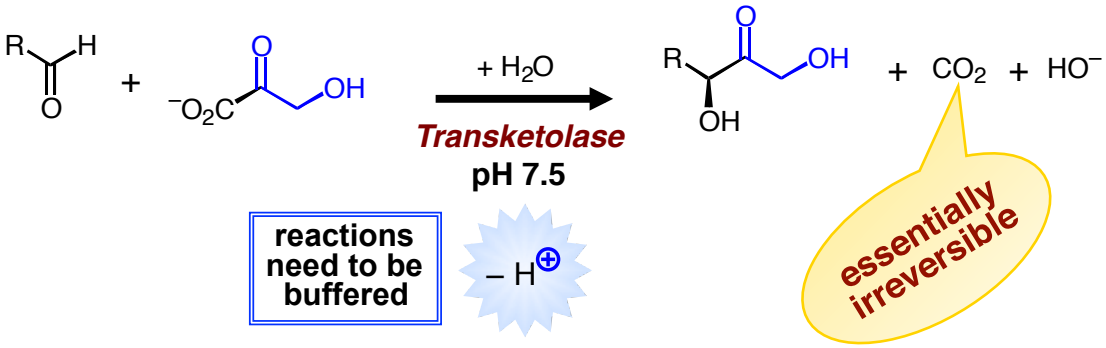
	Isolated yield	ratio OC/α/β
	75%	67/5/34
	76%	54/5/41
	50%	62/4/34
	28%	64/2/34
	25%	66/2/32
	25%	54/5/41
	89%	-/1/99
	82%	-/-/100
	28%	-/-/100
	30%	-/3/97
	45%	-/1/99

Overview

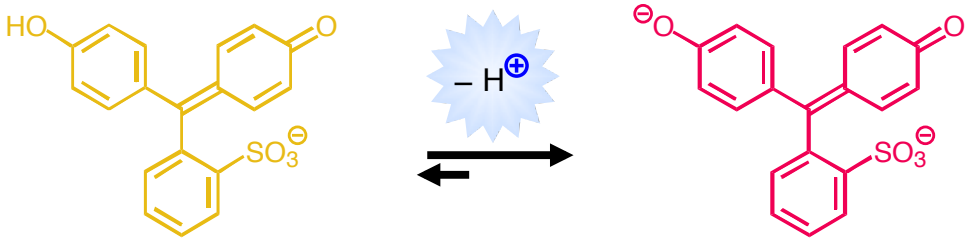
- **Asymmetric Carboligation**
  - Fructose-6-phosphate Aldolase from *E. coli*
  - **Transketolase from *G. stearothermophilus***

New Enzymes: Transketolase



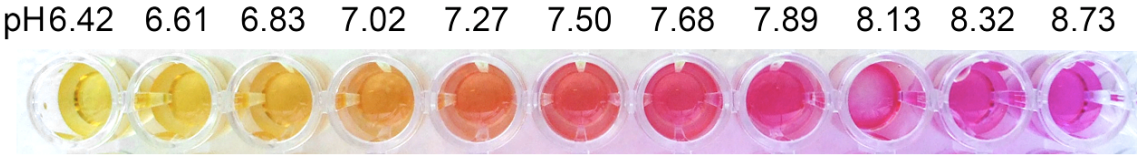


pH Dependent Assay Technology



Phenol Red
neutral form
pK_a 7.4

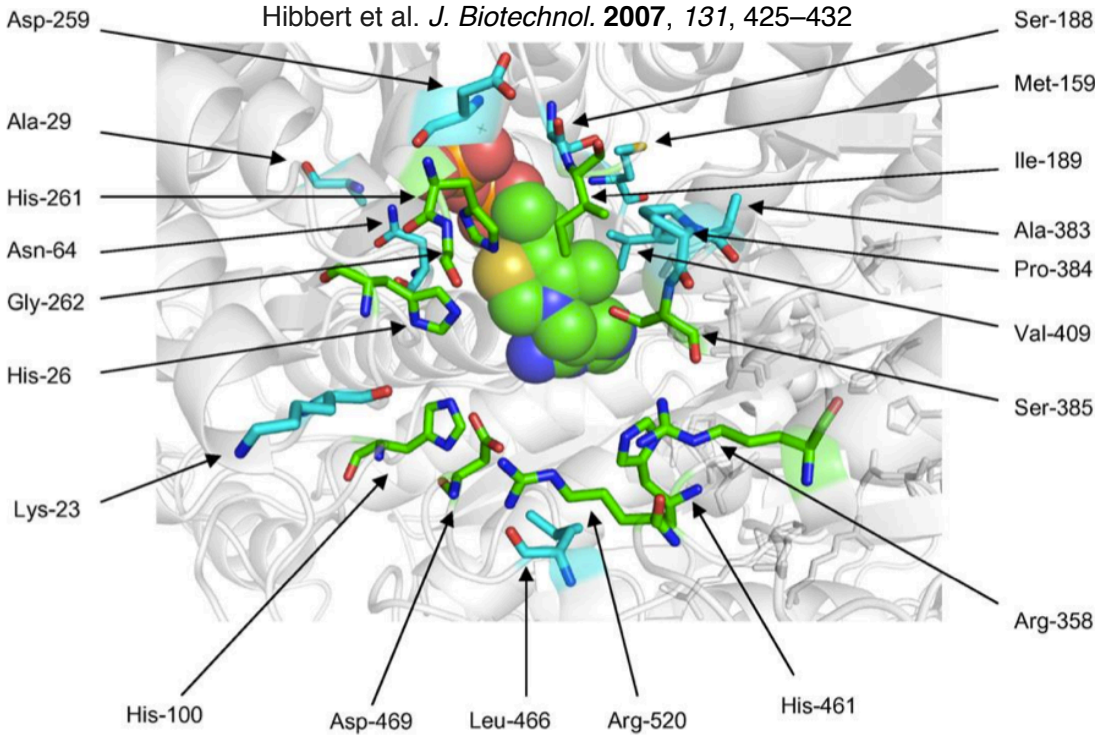
Phenol Red
alkaline form
λ_{max} 557 nm
$\Delta\varepsilon$ 5.6×10^4 $M^{-1}cm^{-1}$



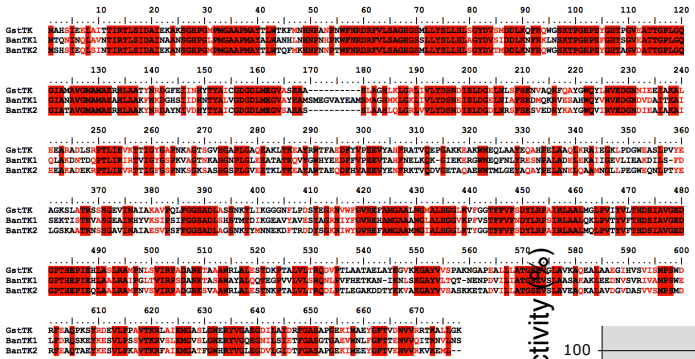
Fessner et al. *ChemBioChem*, **2012**, *13*, 2290–2300

Catalytic Environment

Hibbert et al. *J. Biotechnol.* **2007**, *131*, 425–432



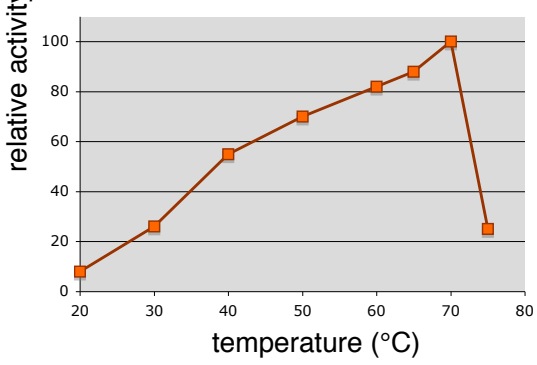
TK from *G. stearothermophilus*



high similarity to transketolase gene from *B. anthracis*

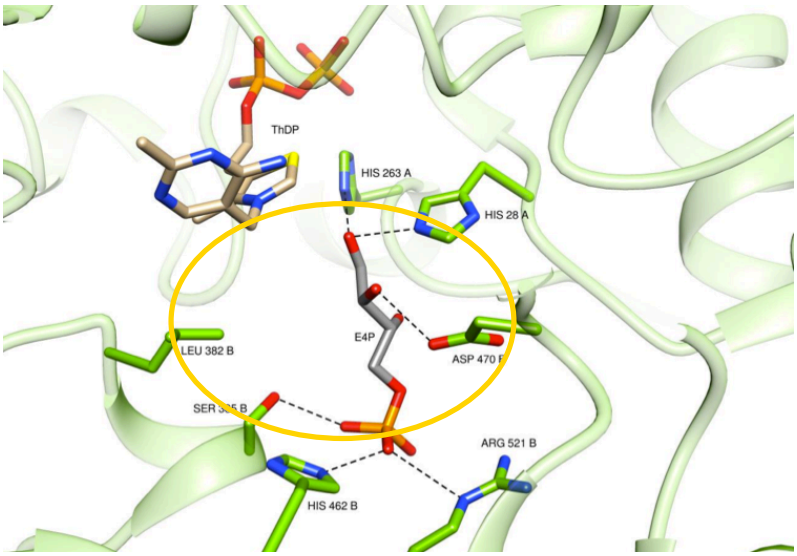
excellent protein over-production in *E. coli*

highly thermostable enzyme activity



Fessner et al. *Adv. Synth. Catal.*, **2013**, *355*, 116–128

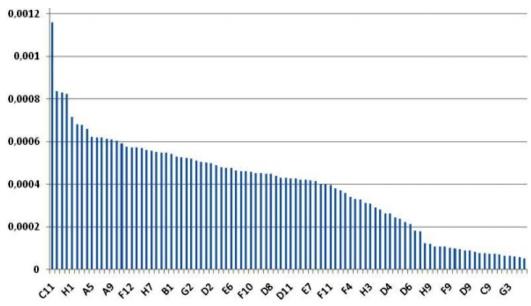
Acceptor Discrimination

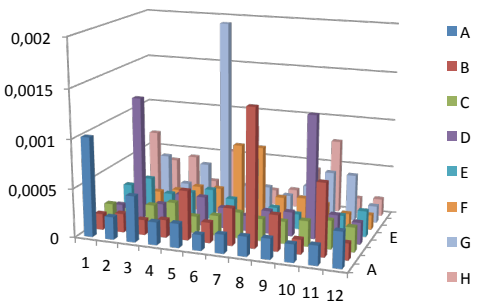


hydrogen bonding contacts to *(R)*-hydroxyaldehydes

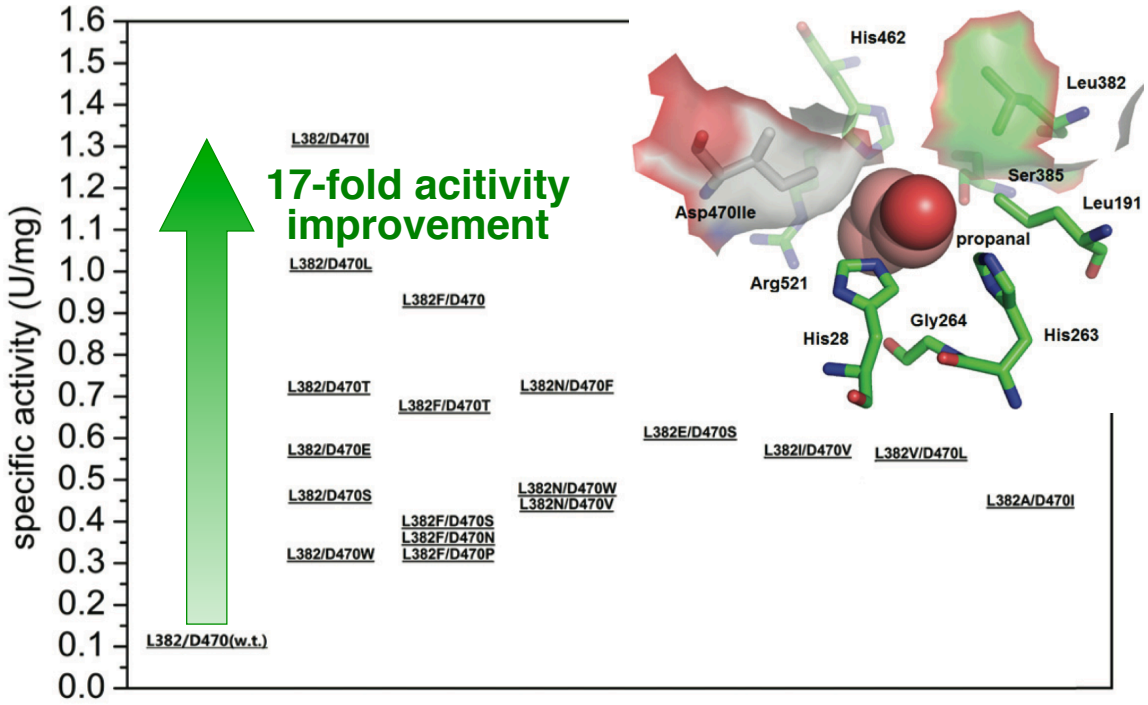
Improved TK Variants

- **structure-guided molecular evolution**
- **focused "small, but smart" libraries**
- **saturation mutagenesis at Leu381/Asp469**
- **residues involved in binding of 2-hydroxyaldehyde**
- **>3,400 colonies (>95% coverage)**
- **screening for propanal / butanal / methoxyethanal**



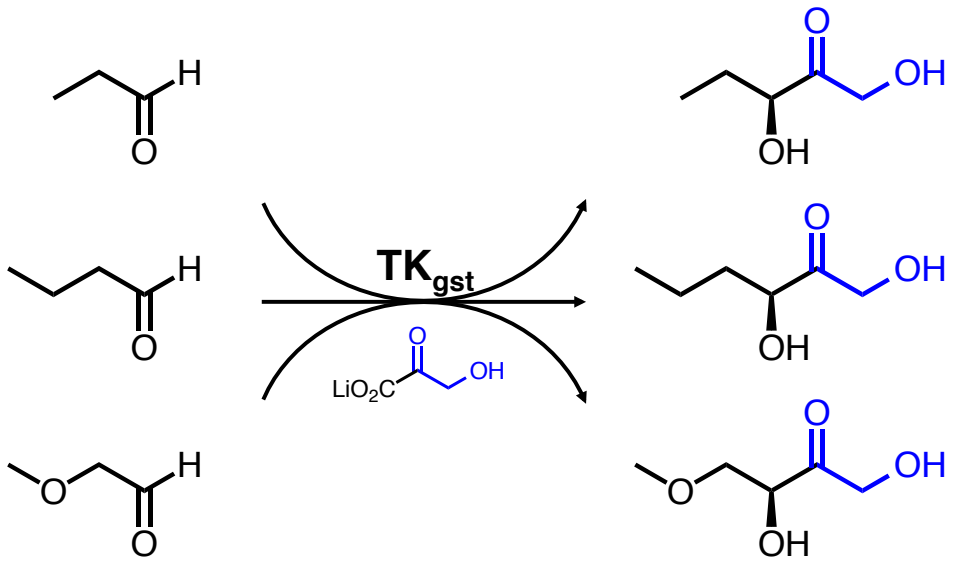


Hydrophobic Substrates



Fessner et al. *manuscript in preparation*

Less Polar Products



Summary

- novel carboligation catalysts
  - thermostable aldolase FSA
  - thermostable transketolase
- broad substrate tolerance
  - engineered donor tolerance for FSA
  - engineered acceptor tolerance for TK
- high enantio- and diastereoselectivity
- one-step creation of structural complexity

Acknowledgments

Dr. Madhura Rale
Deniz Güclü
Sebastian Junker
Thomas Scheidt

Prof. Pere Clapés *(CSIC Barcelona)*
Anna Szekrenyi

Dr. Devamani Titu
Dr. Dong Yi

Prof. Laurence Hecquet *(CNRS Aubiere)*
Dr. Juliane Abdoul Zabar

DFG / CNRS
BMBF / DAAD
EU / COST CM0701
Abbott