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Haibin Chen
Enzymaster (Ningbo) Bio-Engineering Co., Ltd., China, g.kubik@enzymaster.com

Haibin Chen
Enzymaster (Ningbo) Bio-Engineering Co., Ltd.

Yong Koy Bong
Enzymaster (Ningbo) Bio-Engineering Co., Ltd.

Marco Bocola
Enzymaster Deutschland GmbH

Thomas Daußmann
Enzymaster Deutschland GmbH

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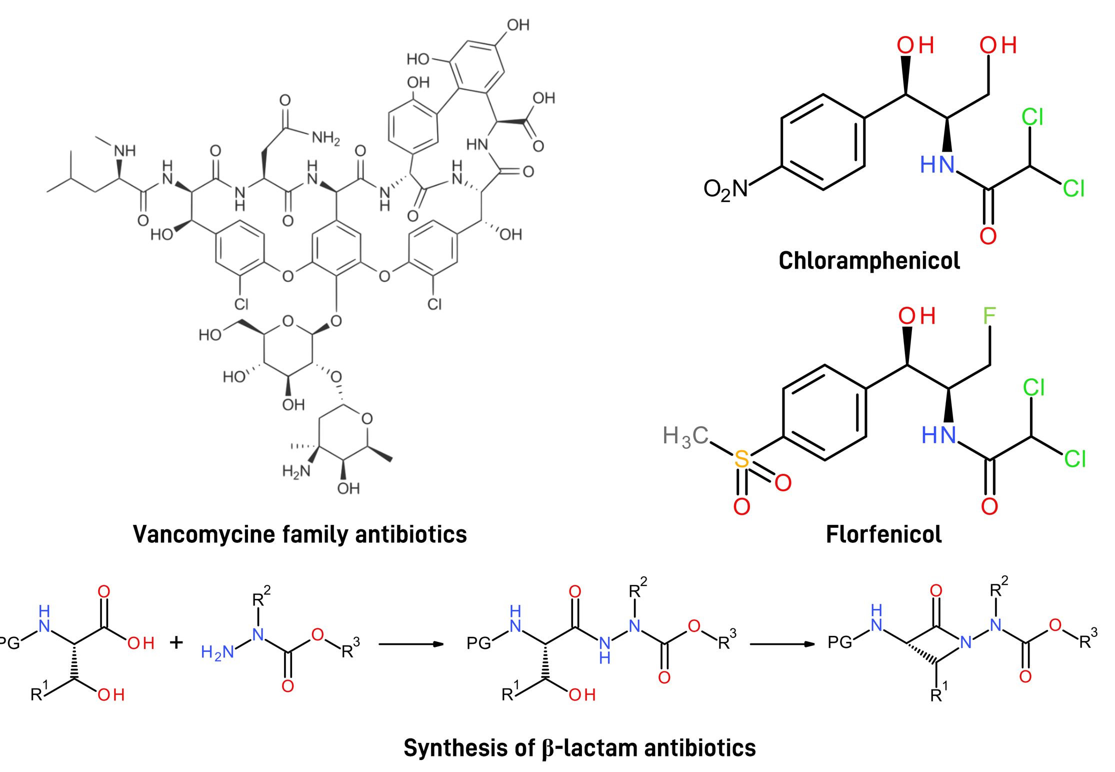
Enzymaster
Green magic happens here

Sustainable Biocatalytic Synthesis of β-Hydroxy-α-Amino Acids on an Industrial Scale

Grzegorz Kubik,[a] Haibin Chen,[b] Marco Bocola,[a] Yong Koy Bong,[b] Thomas Daussmann[a]
a) Enzymaster Deutschland GmbH; b) Enzymaster (Ningbo) Bio-Engineering Co., Ltd.

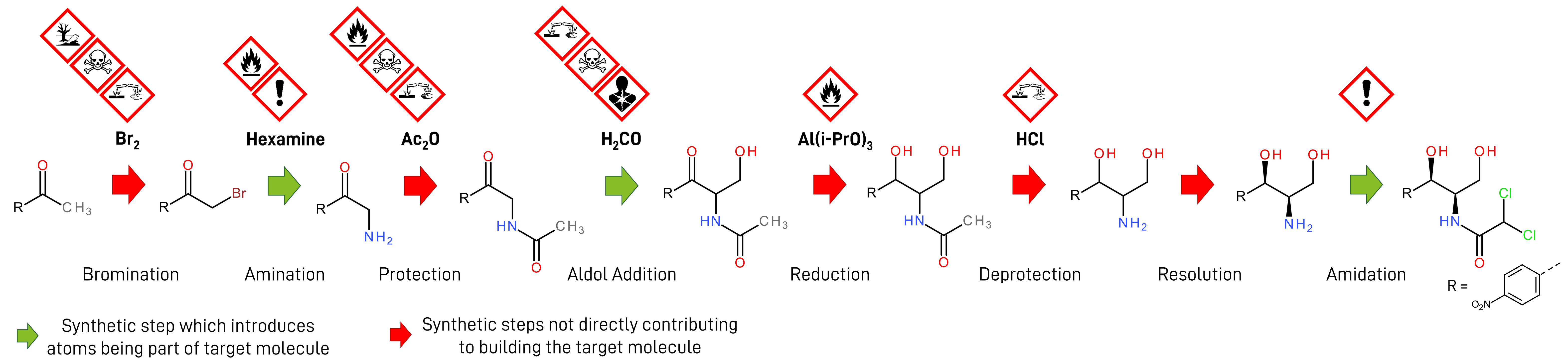


β-hydroxy-α-amino acids are important chiral building blocks in pharmaceutical and fine chemical industry. They can be found as substructure in the Vancomycin antibiotics family and, amongst others, they are intermediates in the synthesis of β-lactam antibiotics as well as Florfenicol. As an industrial case study, we focus on the synthesis of Chloramphenicol, an antibiotic which is for example used for the treatment of penicillin resistant typhus strains.

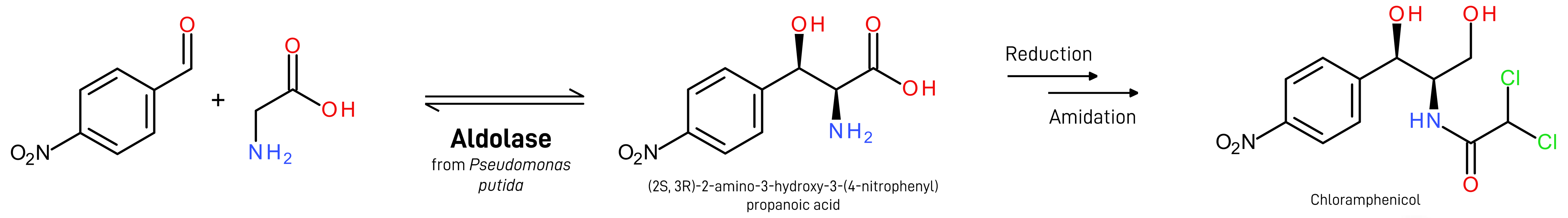
The traditional synthesis of chloramphenicol uses a variety of hazardous chemicals and spans a total of 8 steps, of which only 3 directly contribute to building the molecular scaffold. Thus, a replacement by safe and green biocatalysis is desired.

We reasoned that starting from glycine and *p*-nitro-benzaldehyde, an aldolase enzyme could shorten the synthesis by 5 steps. However, the chosen wild type enzyme was not efficient enough.

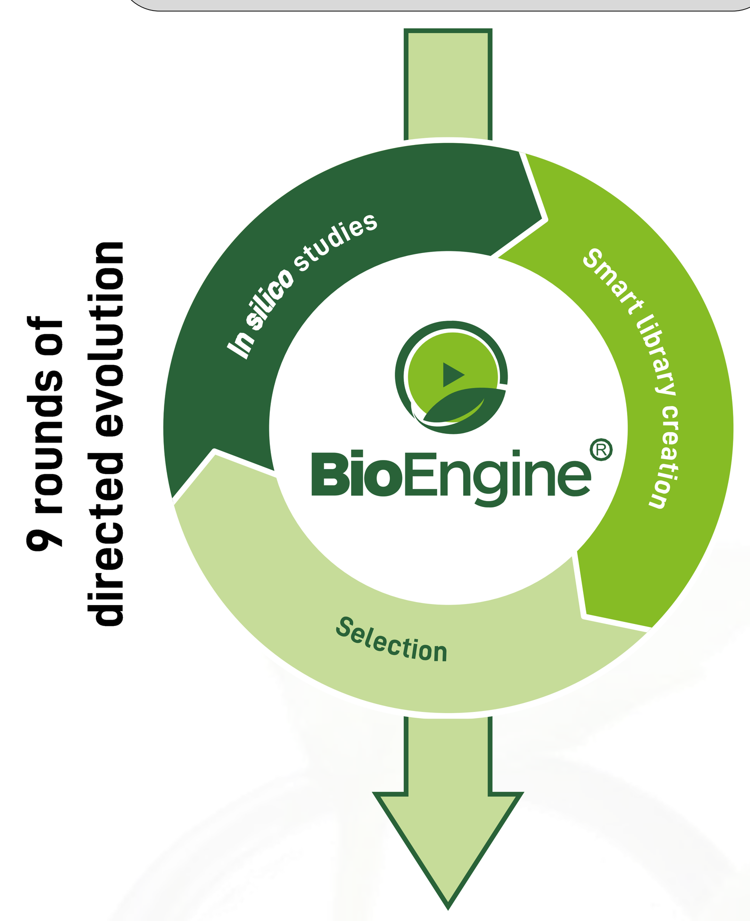
Traditional Chemical Synthesis of Chloramphenicol



Innovative and Environment-Friendly Enzymatic Route

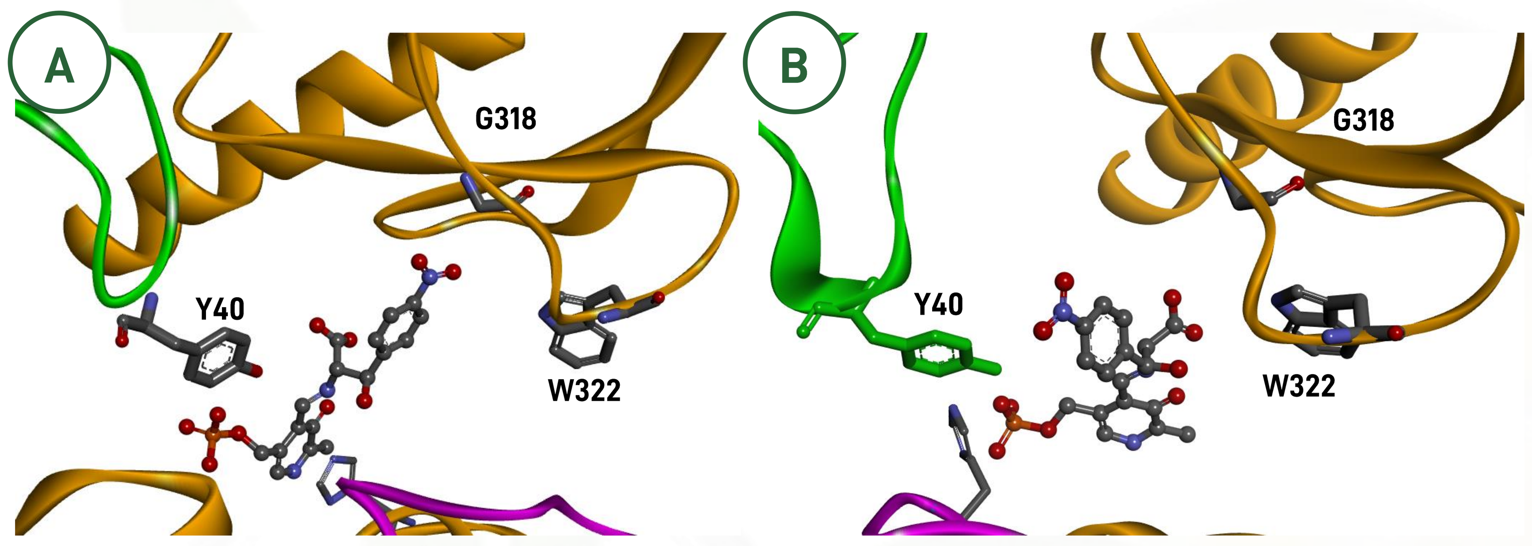


Wildtype Enzyme:
- de 50%
- 10% conversion in 8 h
- 40 g/L substrate load



Industrial Enzyme:
- de >90%
- >80% conversion in 8 h
- 200 g/L substrate load

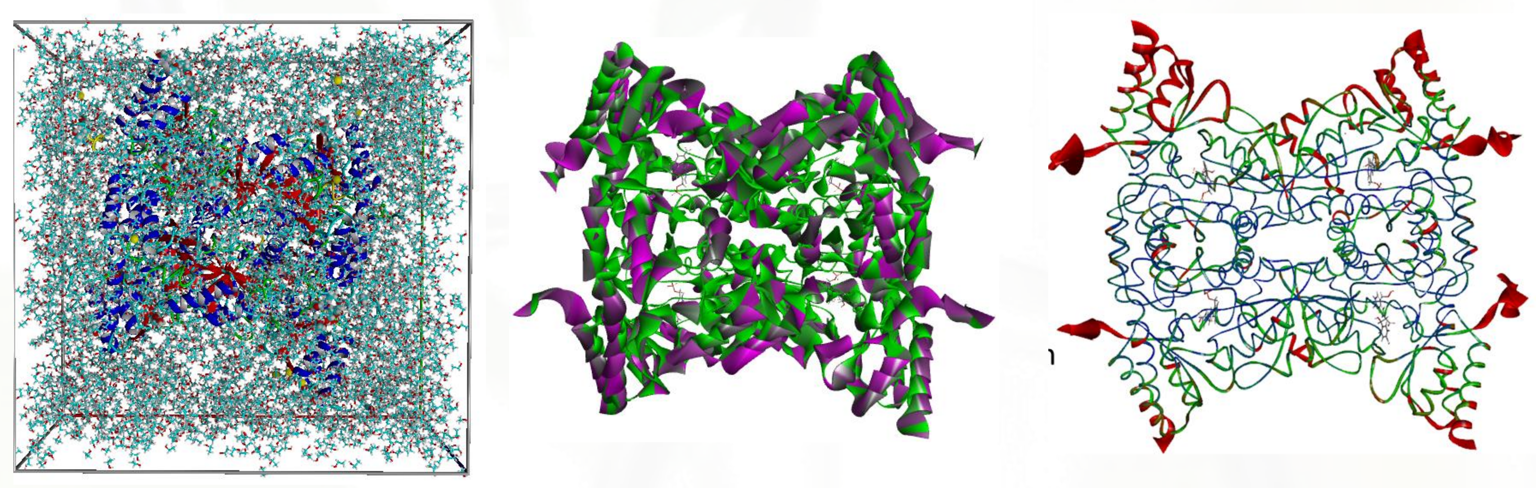
In silico modelling, prescreening and focused library design for smart and efficient enzyme evolution



Benzaldehyde points towards **C-terminal** domain — Benzaldehyde points towards **N-terminal** domain

Only the conformation of the external aldimine intermediate where the benzaldehyde points towards the C-terminal domain (A) leads to the desired 2*S*-3*R* -diastereomer.
In consequence, we **predicted mutations at hotspots in the C-terminus** and contacting domains, which stabilize this conformation in MD-simulations.
Based on these results we created smart enzyme variant libraries for fast and efficient experimental screening.

MD-simulations under reaction conditions to analyze destabilizing organic solvent contacts



60 Vol% Water 40 Vol% EtOH — 21179 Residues 4480 Residues — Solvent contact analysis — RMSF analysis

To allow for high substrate loads, the use of high ethanol concentrations is needed, which is not tolerated by the wildtype aldolase. MD-simulations under the desired process conditions allowed us to **identify sites**, in the C-terminus and at tetramer interfaces, **which show high mobility induced by solvent contacts.** Screening of enzyme libraries targeting these sites yielded in an enzyme variant with strongly improved stability towards 40% ethanol and improved productivity.

BioEngine® allowed us to train the aldolase enzyme to produce the desired β-hydroxy-α-amino acid with good diastereoselectivity and high activity at high substrate load and ethanol concentration. This final enzyme variant now meets the industrial targets for sustainable production at multi-ton scale.

This example demonstrates how computer-aided directed enzyme evolution enables the development of superior processes which are defined by reduced numbers of synthetic steps, milder and safer reaction conditions, and lower environmental impact.

References:
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Contact:
a) **Enzymaster Deutschland GmbH**
Neusser Str. 39, 40219 Düsseldorf, Germany.
Tel.: +49(0) 211-15821610
Web: www.enzymaster.de
E-mail: info@enzymaster.de

b) **Enzymaster (Ningbo) Bio-Engineering Co., Ltd.**
5th Floor, 2nd Block, Huadongcheng Building,
333 North Century Avenue, Ningbo, China
Tel.: +86-574-87817737
Web: www.enzymaster.com
E-mail: info@enzymaster.com

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