BIOCATALYSIS FOR THE SUSTAINABLE SYNTHESIS OF SMALL MOLECULES INTERMEDIATES FOR APIs

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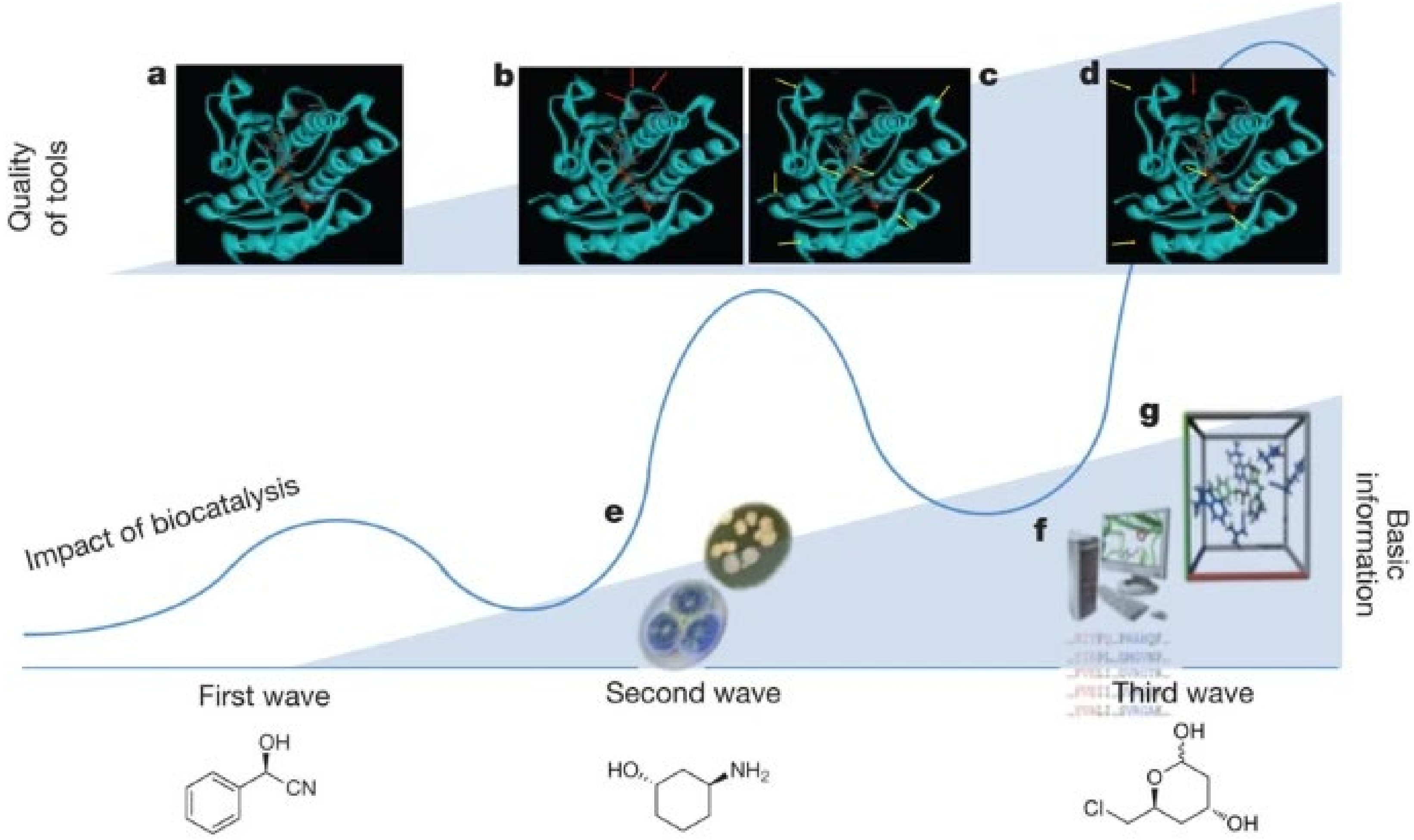
THE "WAVES" OF BIOCATALYSIS



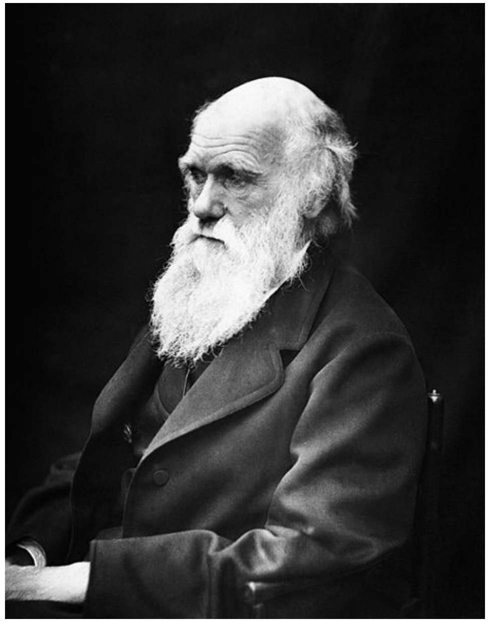
Reproduced from *Nature* **2012**, 485, 185-194.

The fourth wave is now!

4th wave:
Ultra-high-throughput screening,
AI and machine learning,
Metagenomics and advanced genome editing,
New materials

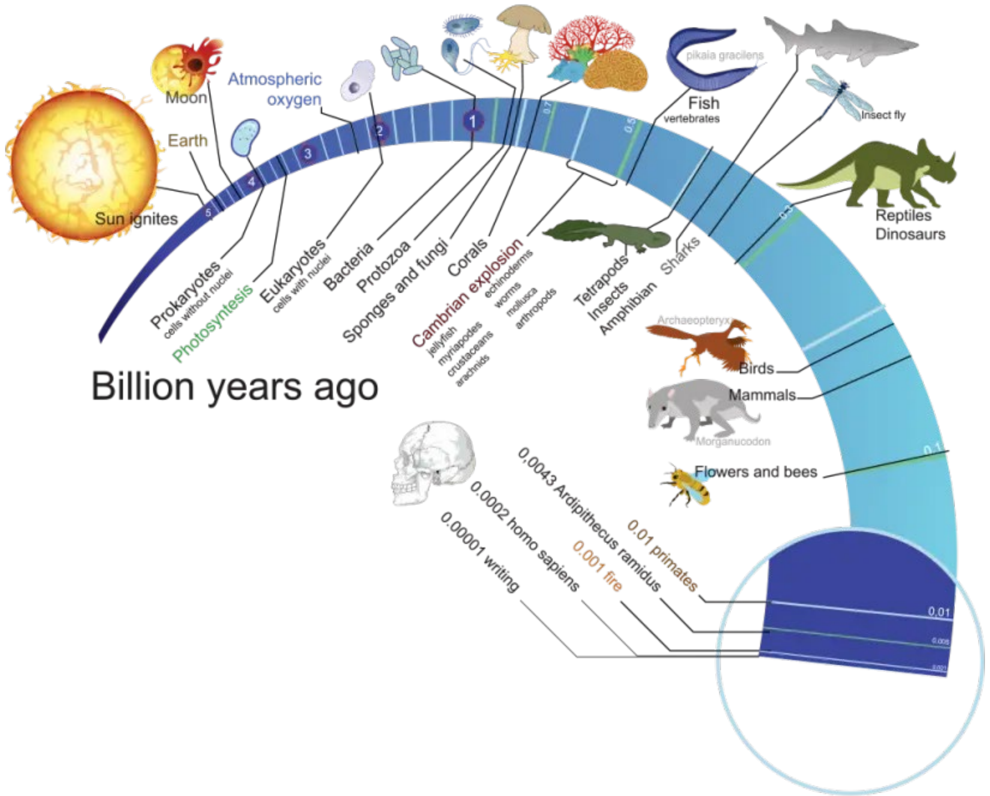
1st wave: >100 years ago, Use of microbial strains, plant or animal tissues ("black box" biocatalysis)
2nd wave: 1980s, Revolution in gene technology (recombinant enzyme expression, site-directed mutagenesis, enzyme immobilization, etc.)
3rd wave: 1990s, Enzyme (protein) engineering (directed evolution, bioinformatics, computer modelling)

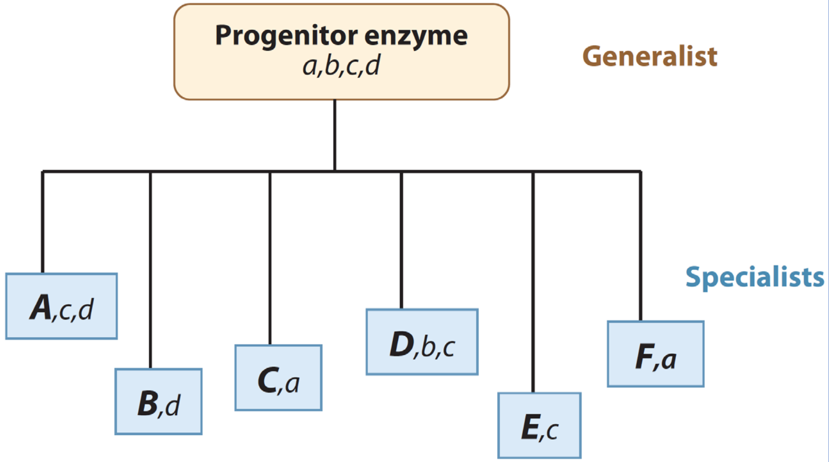
Directed evolution (Iterative process like natural evolution)



Charles Darwin

1809 - 1882





Divergence of new enzyme family members in nature

DIRECTED EVOLUTION OF ENZYME: A NON-RATIONAL ENZYME ENGINEERING METHOD

Iterative process (like natural evolution): the best mutated enzymes are used as a template for the next generation of further improved enzymes

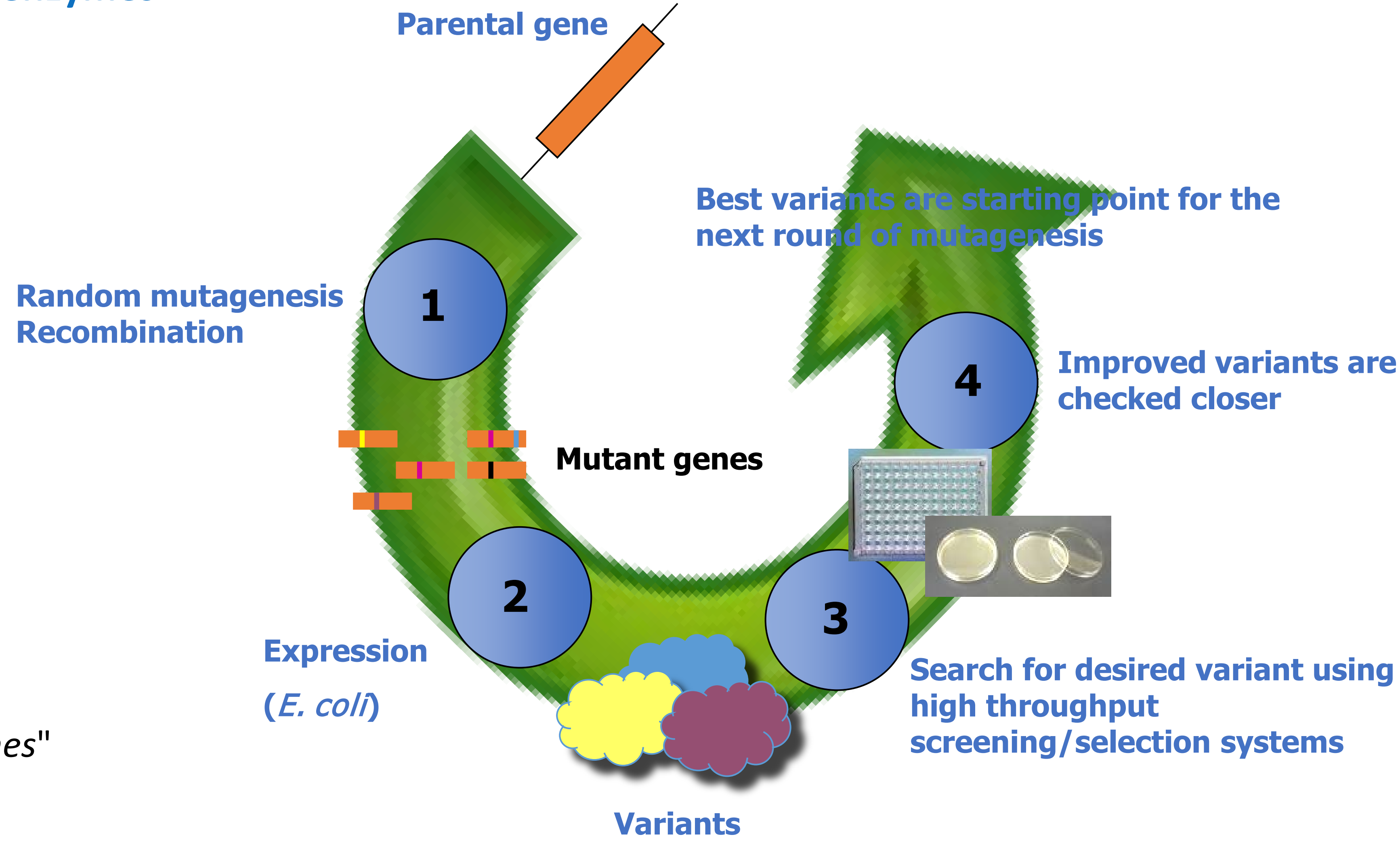
Frances H. Arnold

The Nobel Prize Chemistry 2018

Prize motivation:
"For the directed evolution of enzymes"

Prize share: ½

(shared with G. P. Smith & G. P. Winter)



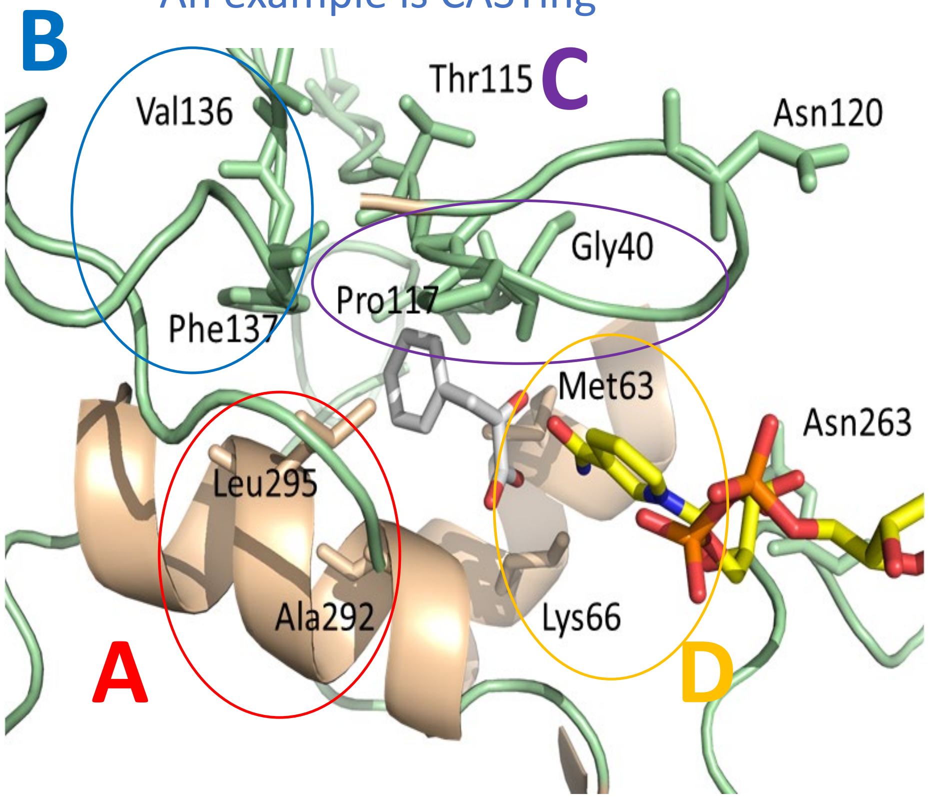
Collapse the time of natural evolution from millions of years to months or weeks

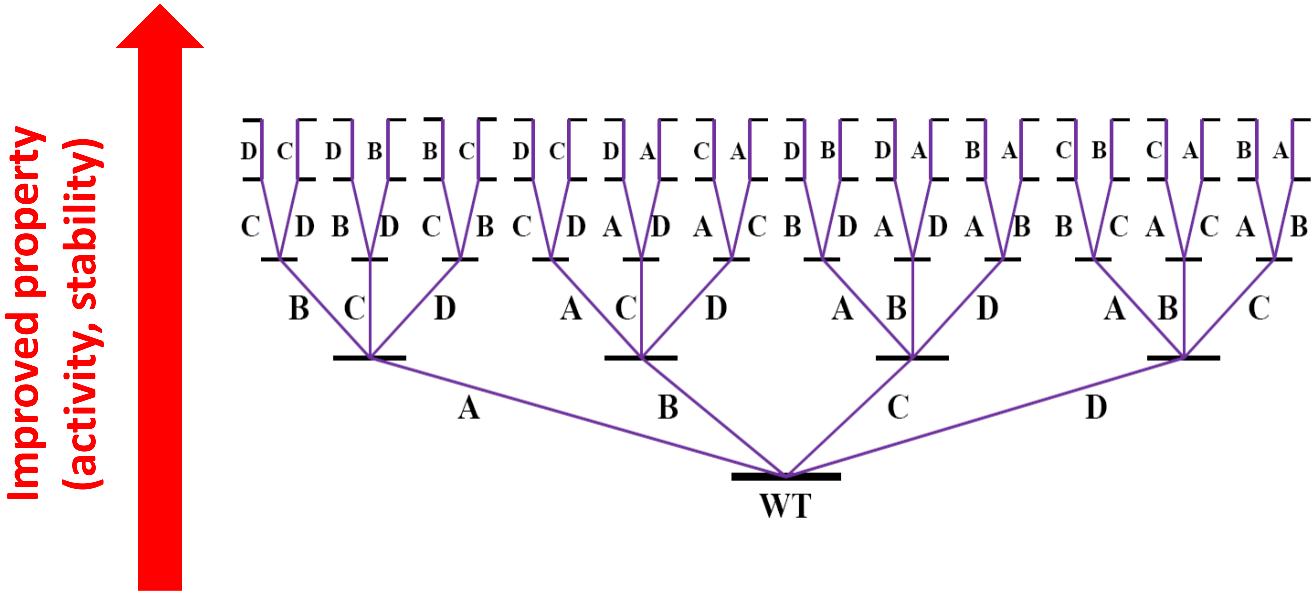
STRUCTURE-GUIDED ENZYME ENGINEERING (A SEMI-RATIONAL APPROACH)

It tries to combine the best between directed evolution and site directed mutagenesis.

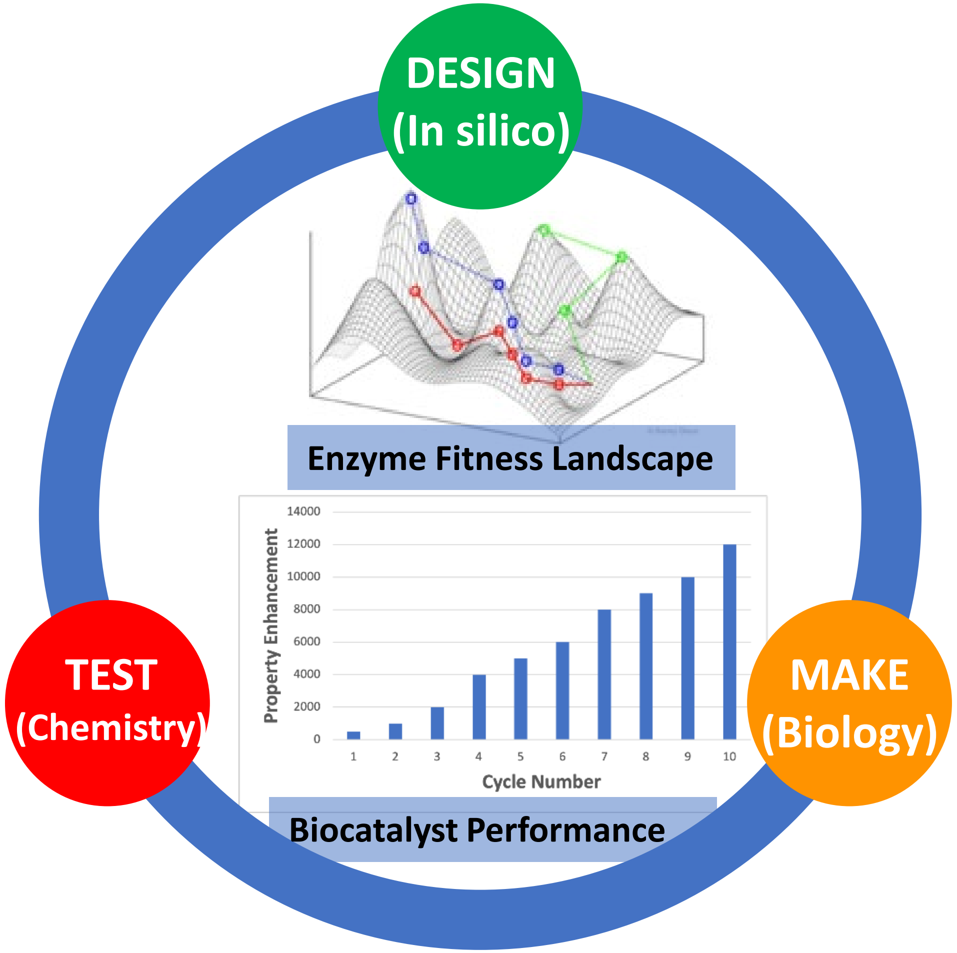
At least, knowleadge of enzyme 3D structure is required.

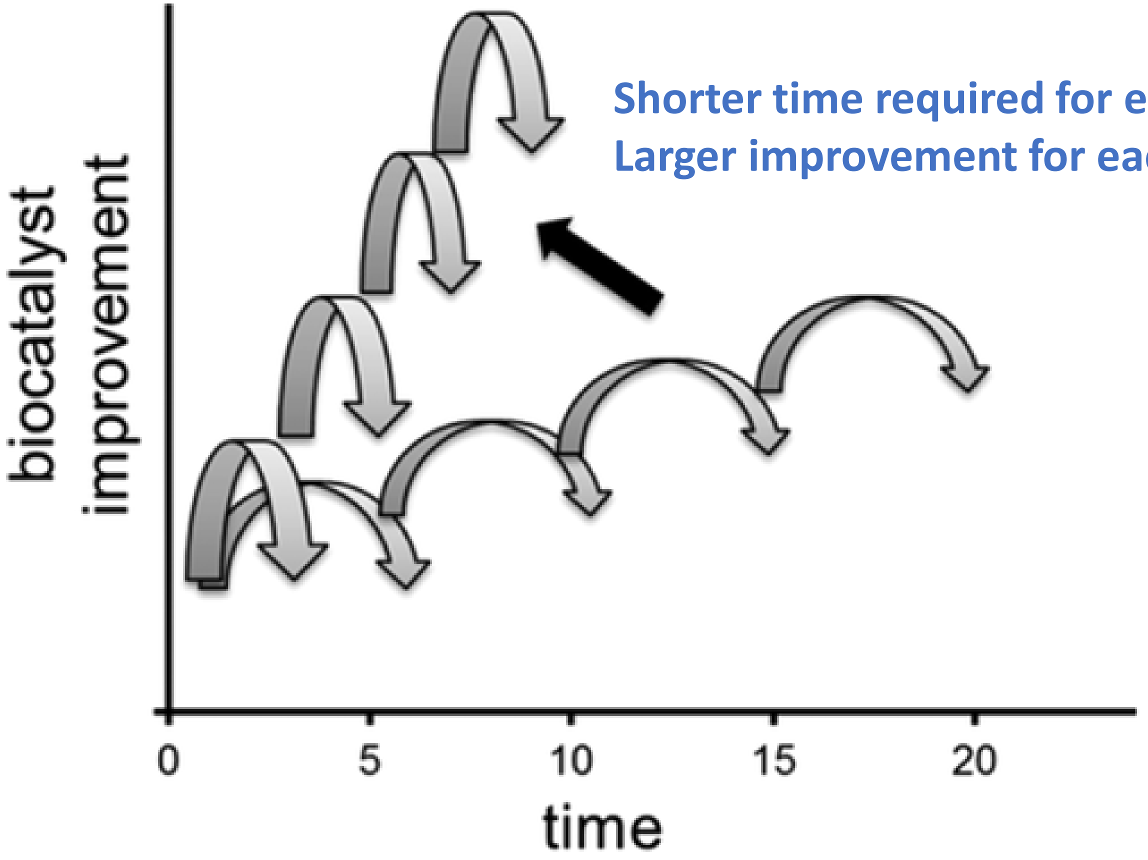
An example is CASTing





- **Reducing the time** (and costs) needed to engineer a biocatalysts
- **Expand the number** of enzymatic reactions that can be used for chemical synthesis
- **Create** new-to-nature enzymatic activities



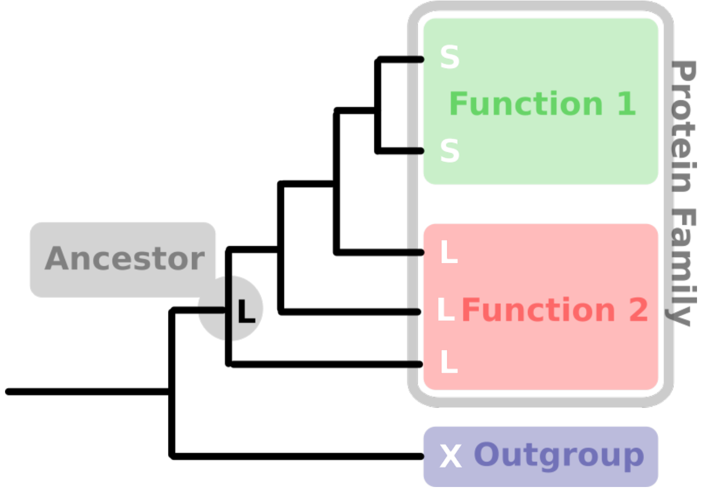


Shorter time required for each cycle
Larger improvement for each cycle

The 4th wave of biocatalysis: Ancestral sequence reconstruction (ASR) and Bioinformatics

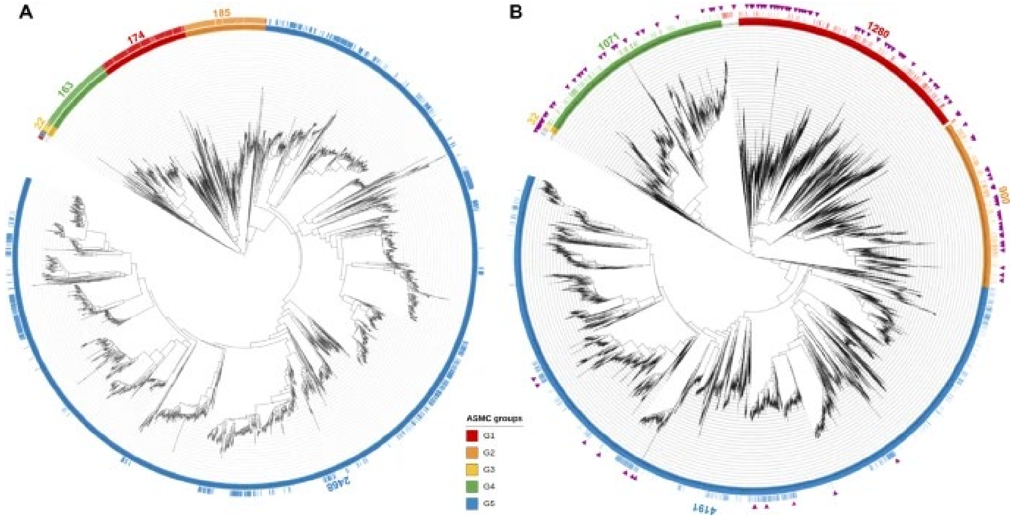
Ancestral sequence reconstruction

Early life on Earth was more hostile to microorganisms
(Hypothesis: promiscuous but also more stable enzymes?)

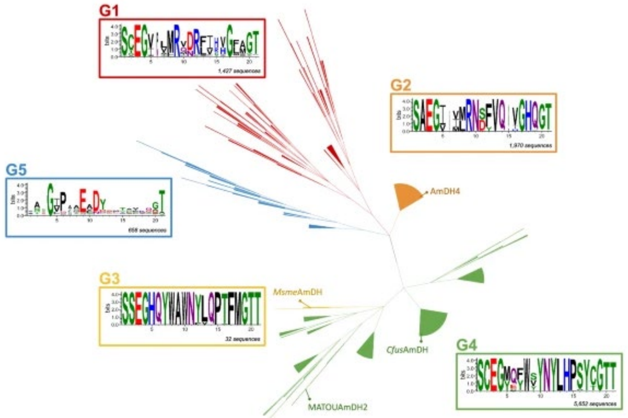


Bioinformatics, metagenomics

Example: the metagenomic sequence space of amine dehydrogenases



Sequence analysis and clustering



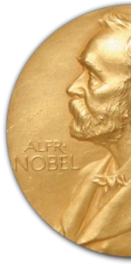
THE 4TH WAVE OF BIOCATALYSIS: ADVANCED COMPUTATIONAL TOOLS, AI AND ML

The Nobel Prize Chemistry 2024

David Baker

Demi Hassabis **John M. Jumper**

(aff.: Google DeepMind)



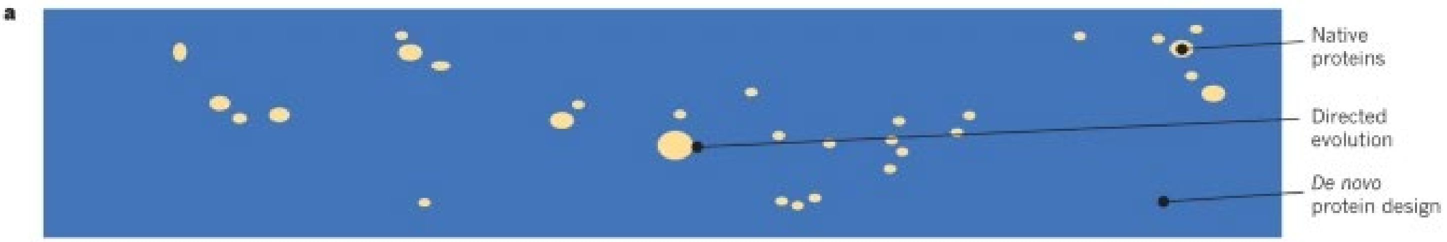
Prize share: ½

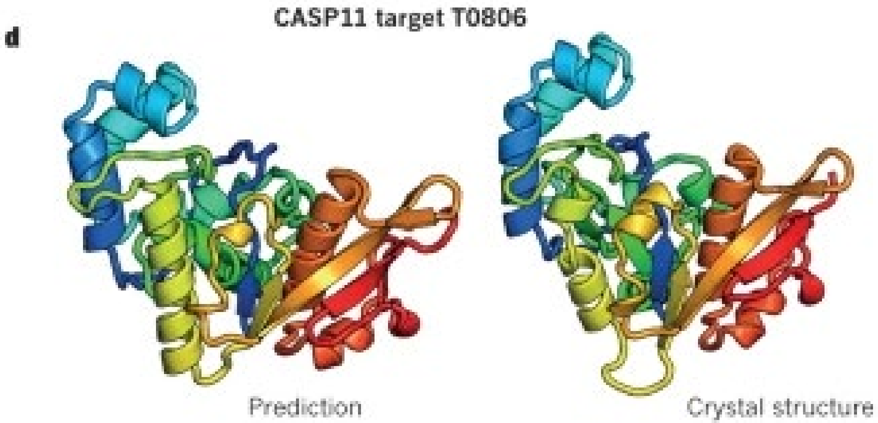
Prize motivation:
"for computational protein design"

Prize share: ¼ each

Prize motivation:
"for protein structure prediction"

Computational *de novo* protein design





The Nobel Prize Chemistry 2024



David Baker **Demi Hassabis** **John M. Jumper**

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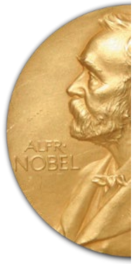


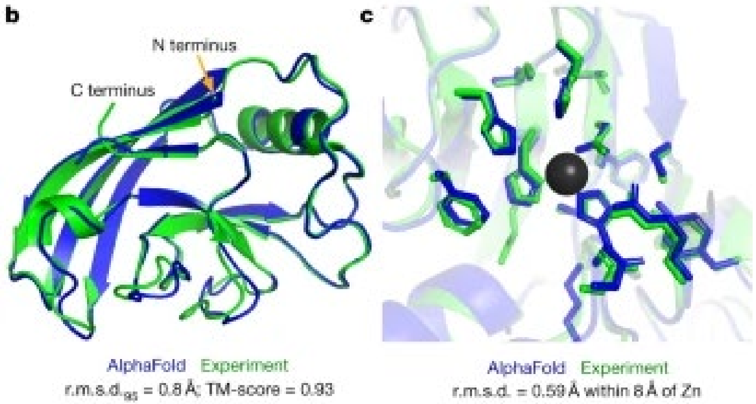

Prize share: ½

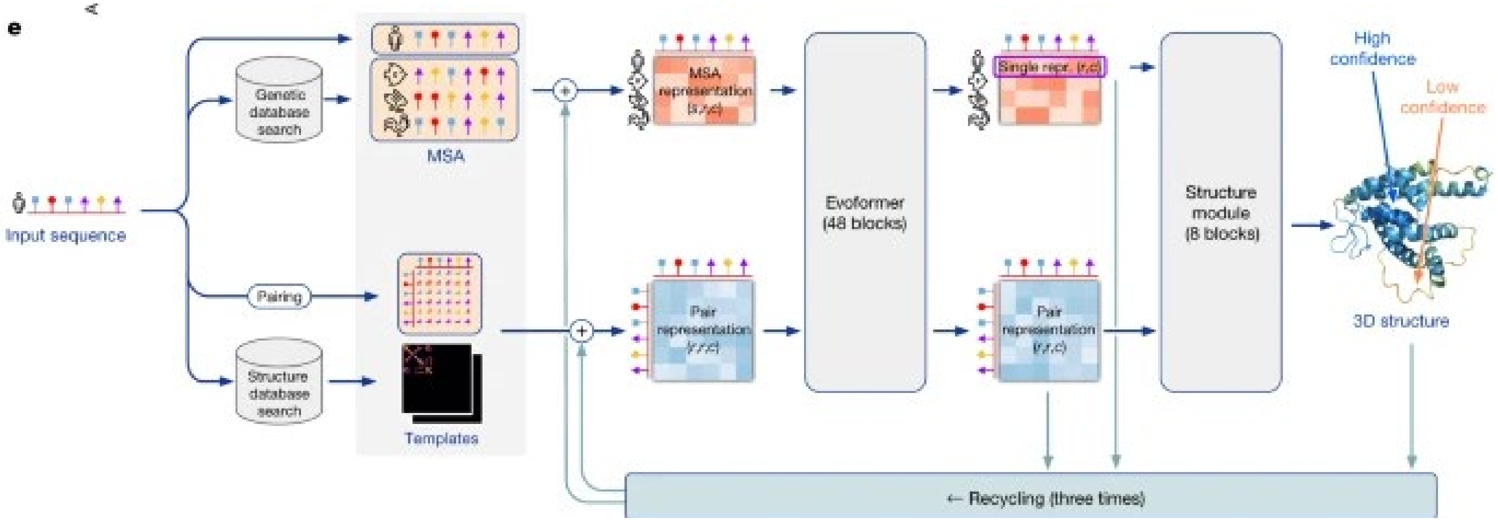
Prize motivation:
“for computational protein design”

Prize share: ¼ each

Prize motivation:
“for protein structure prediction”

AlphaFold: an ML-, neural network-based method to predict protein structures with atomic accuracy **even when no similar structure is known**





THE 4TH WAVE OF BIOCATALYSIS: ADVANCED COMPUTATIONAL TOOLS, AI AND ML

The Nobel Prize Chemistry 2024



David Baker **Demi Hassabis** **John M. Jumper**

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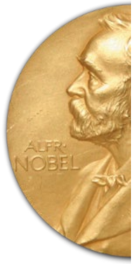


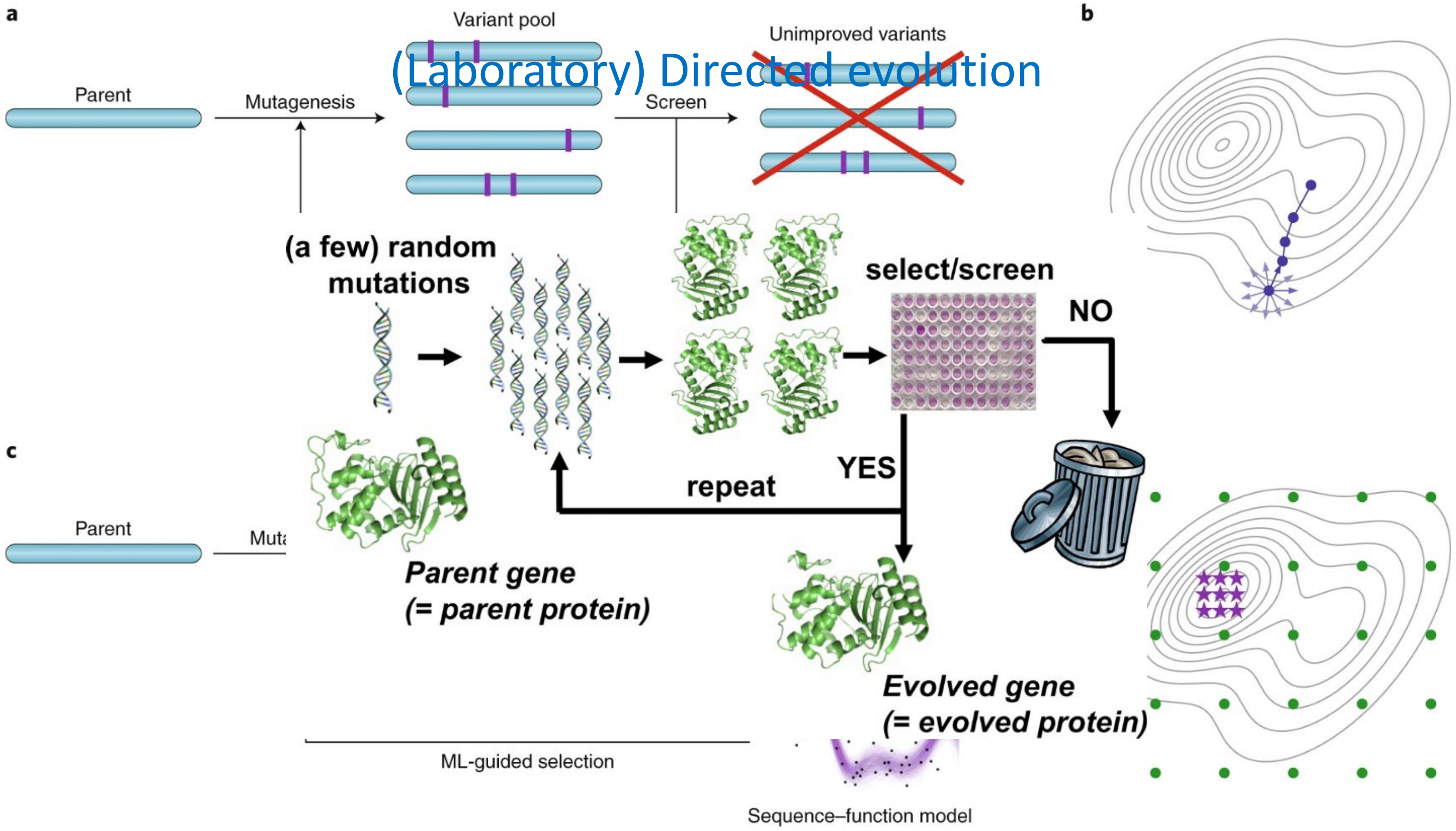
Prize share: ½

Prize motivation:
"for computational protein design"

Prize share: ¼ each

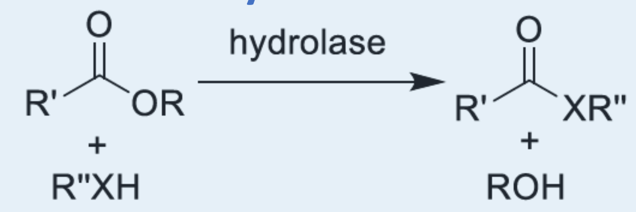
Prize motivation:
"for protein structure prediction"

Comparing directed evolution with and without machine learning



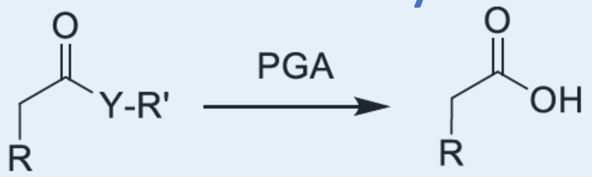
Most commonly used biocatalytic reactions in the pharmaceutical industry (descending order)

Hydrolases



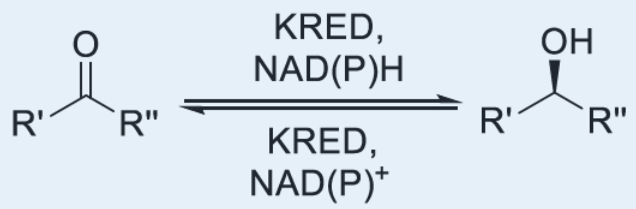
R"XH = water, alcohol, 1° and 2° amine

Penicillin G acylase

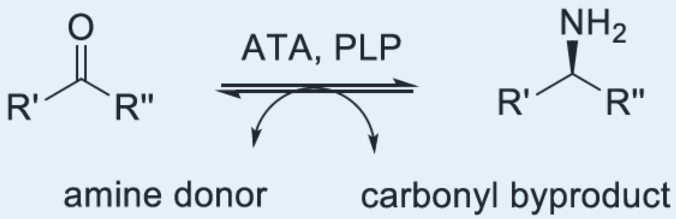


R = Ph, OPh
Y = O, NH
R' = variable

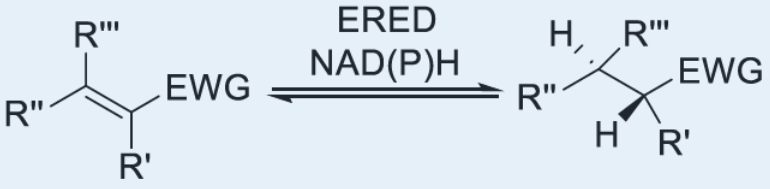
Alcohol dehydrogenases



Transaminases



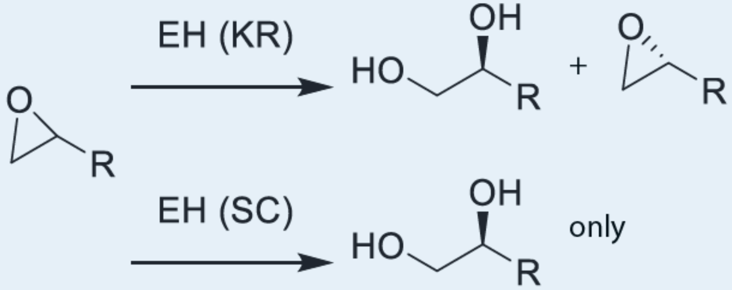
Ene-reductases



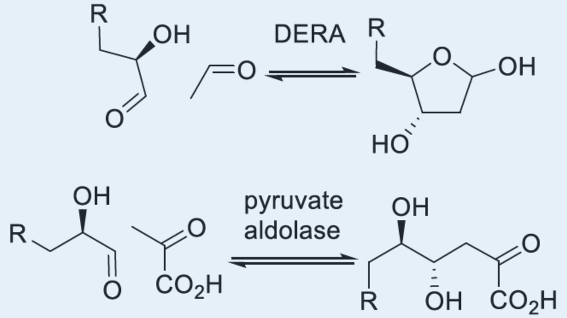
EWG : NO_2 > CHO > COR > CO_2R >CN

Nitrilases

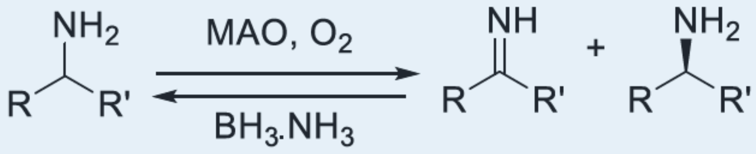
Epoxide hydrolase



Aldolases

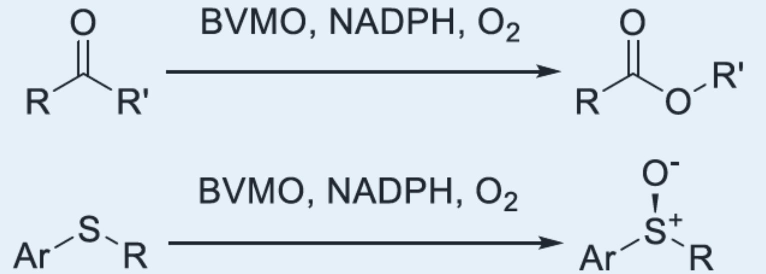


Amine oxidases

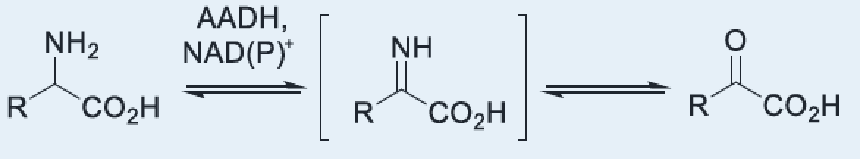


MOST COMMONLY USED BIOCATALYTIC REACTIONS IN THE PHARMACEUTICAL INDUSTRY

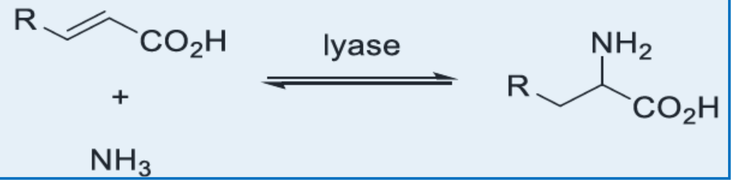
Baeyer-Villiger monooxygenases



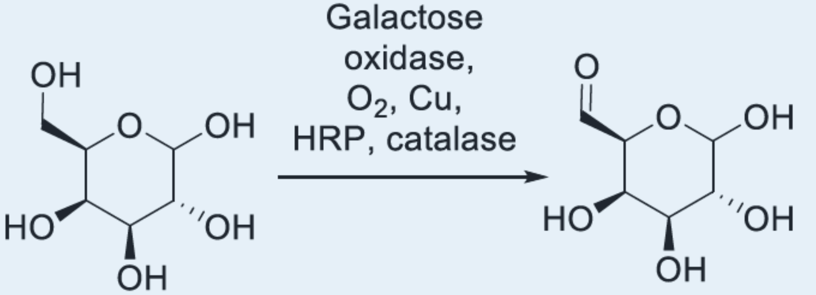
Amino acid dehydrogenases



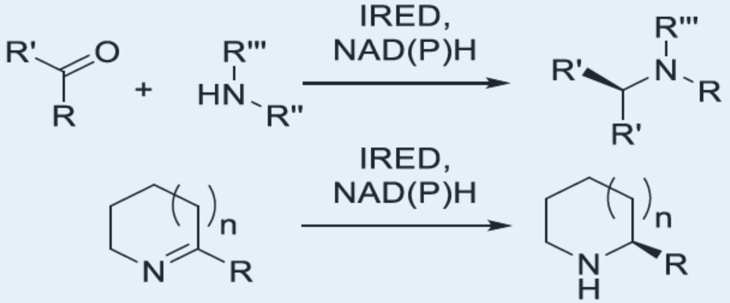
Ammonia lyases



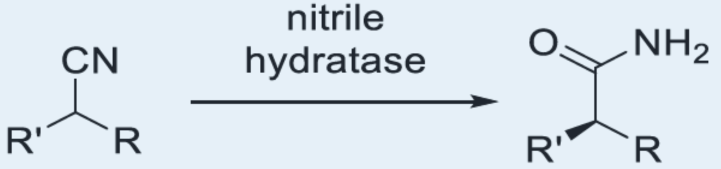
Alcohol oxidases



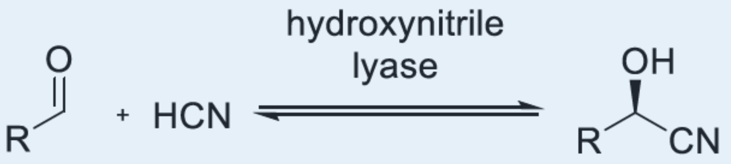
Imine reductases



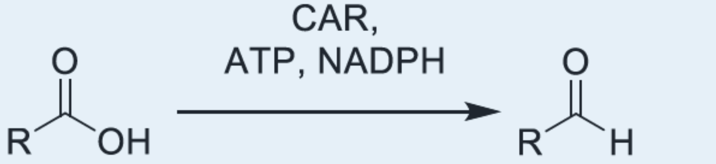
Nitrile hydratases



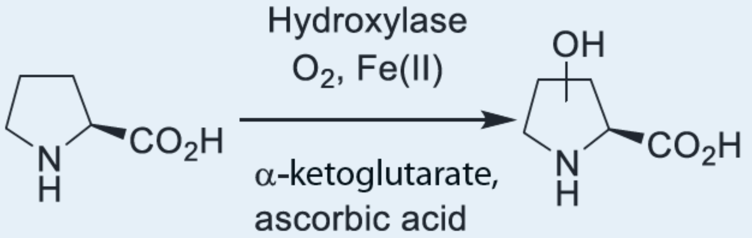
Hydroxynitrile lyases



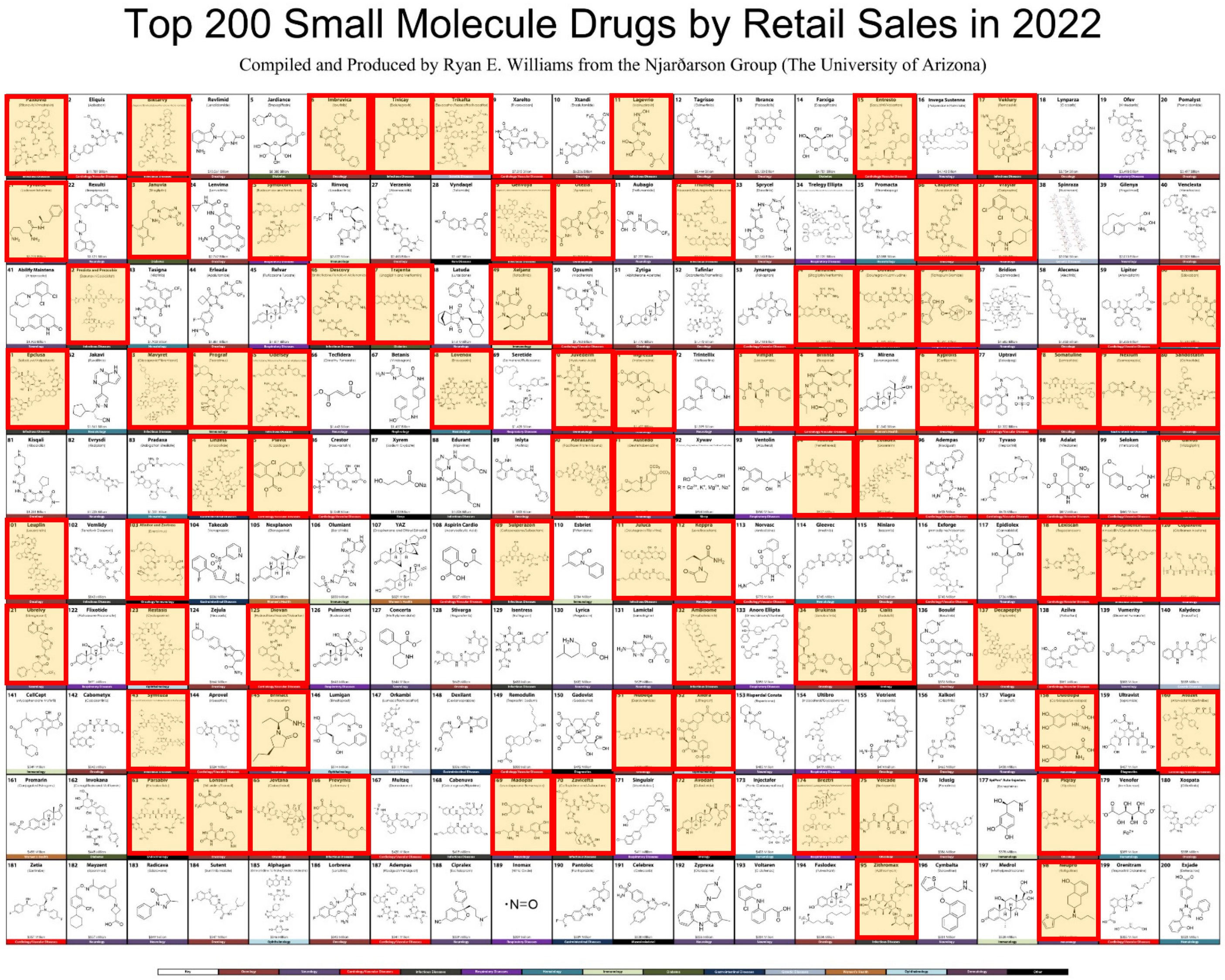
Carboxylic acid reductases

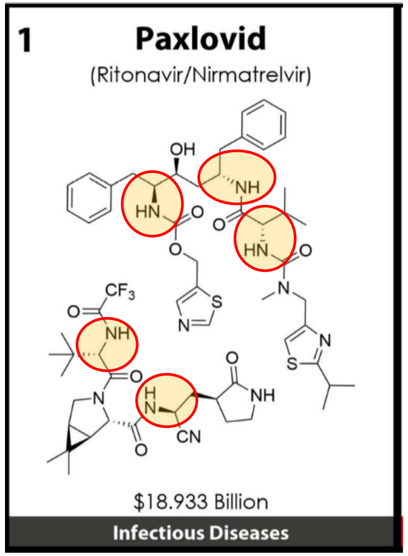


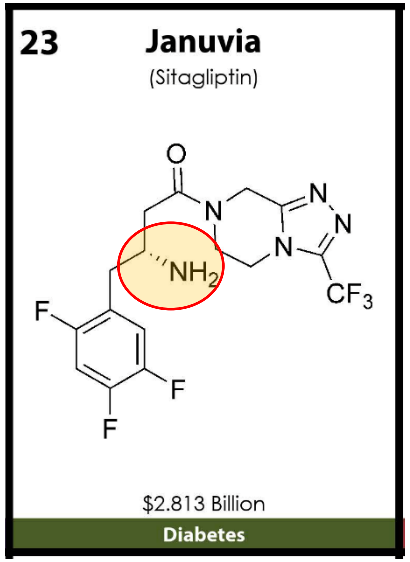
Hydroxylases



SYNTHESIS OF CHIRAL AMINES AS INTERMEDIATES FOR APIs



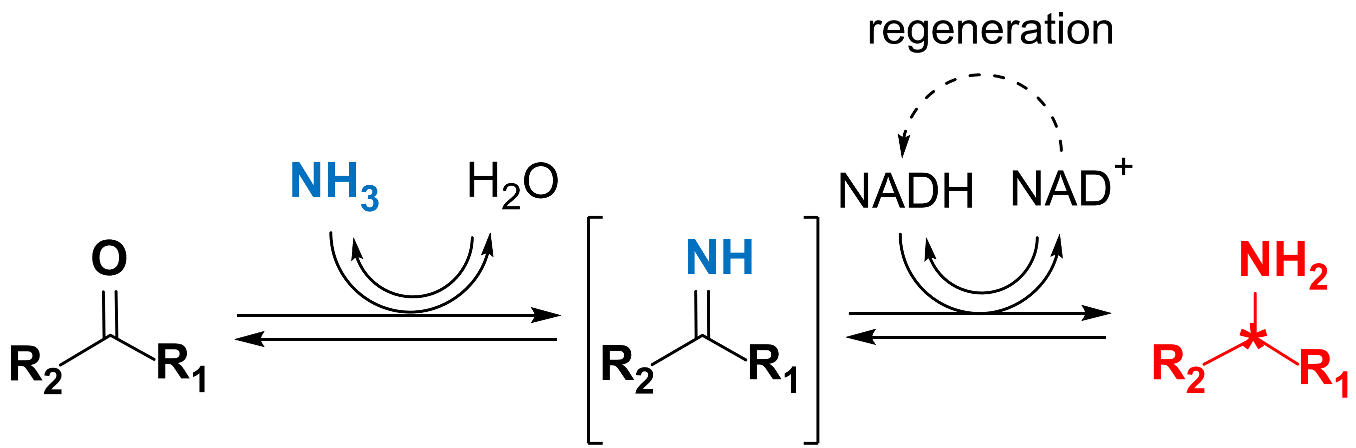




77 out of the "Top 200 Small Molecules" contain an α-chiral amine moiety

Compiled by Njardarson Group (The University of Arizona)

AN EXAMPLE FROM OUR RESEARCH: SYNTHESIS OF α-CHIRAL AMINES



APIs

Biocatalytic methods using **amine dehydrogenases** (AmDHs)

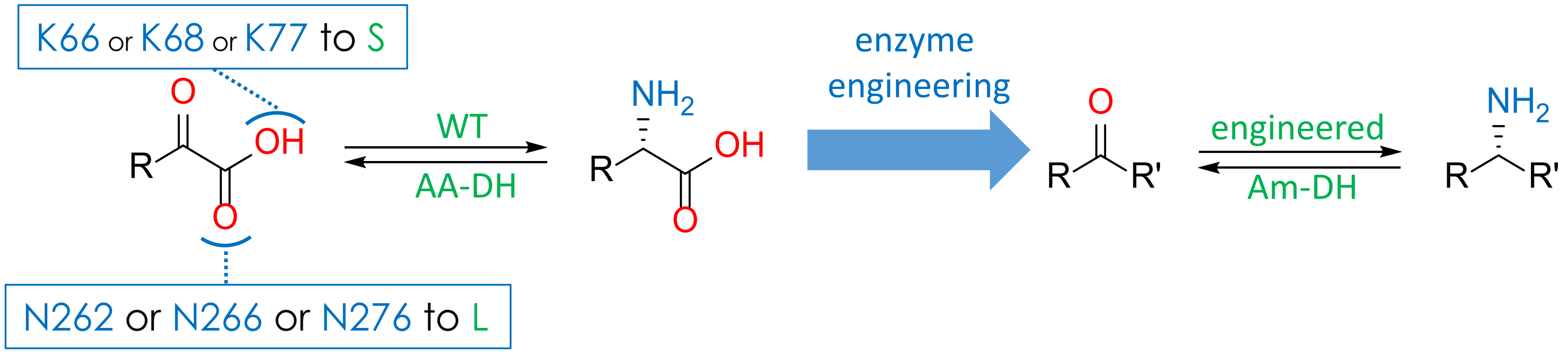
(i) one-step synthesis of α-chiral amines through the reductive amination of prochiral carbonyl-compounds

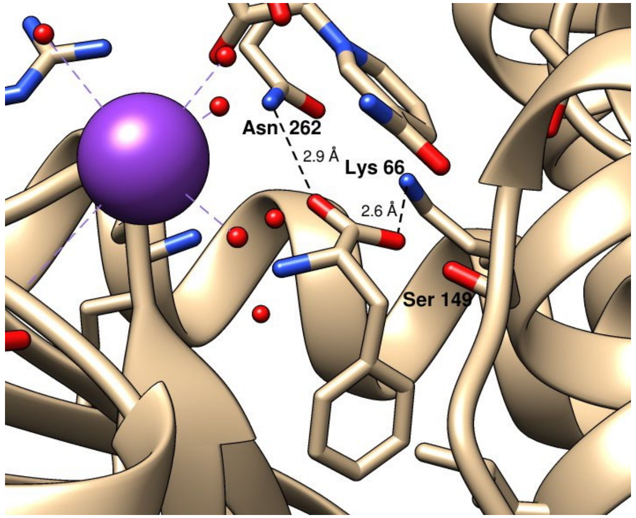
(ii) mild reaction conditions

(iii) chiral amines with an excellent optical purity are obtained

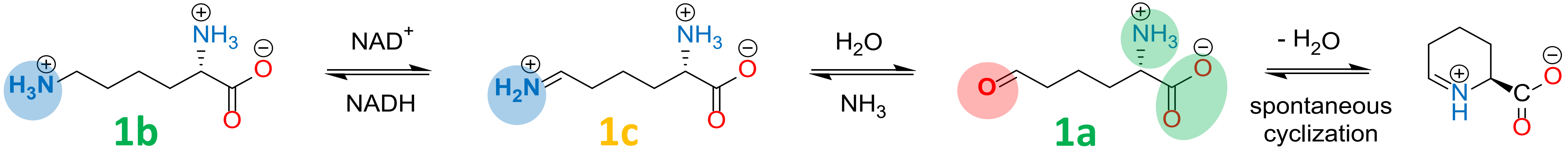
ASYMMETRIC REDUCTIVE AMINATION USING AMINE DEHYDROGENASES (AmDHs)

From L-α-amino acid dehydrogenases to give "(*R*)-selective AmDHs" (Bommarius)





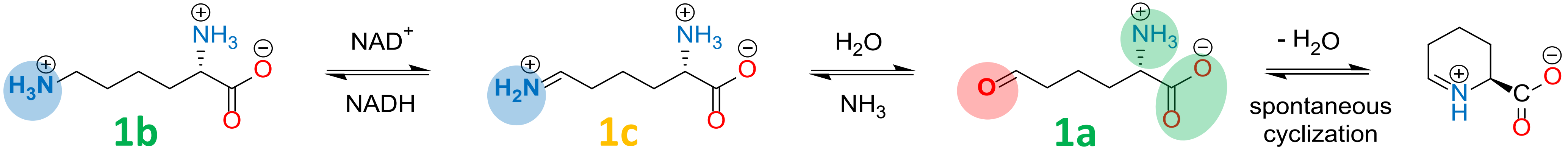
From ε-L-lysine dehydrogenase (LysEDH) to AmDHs



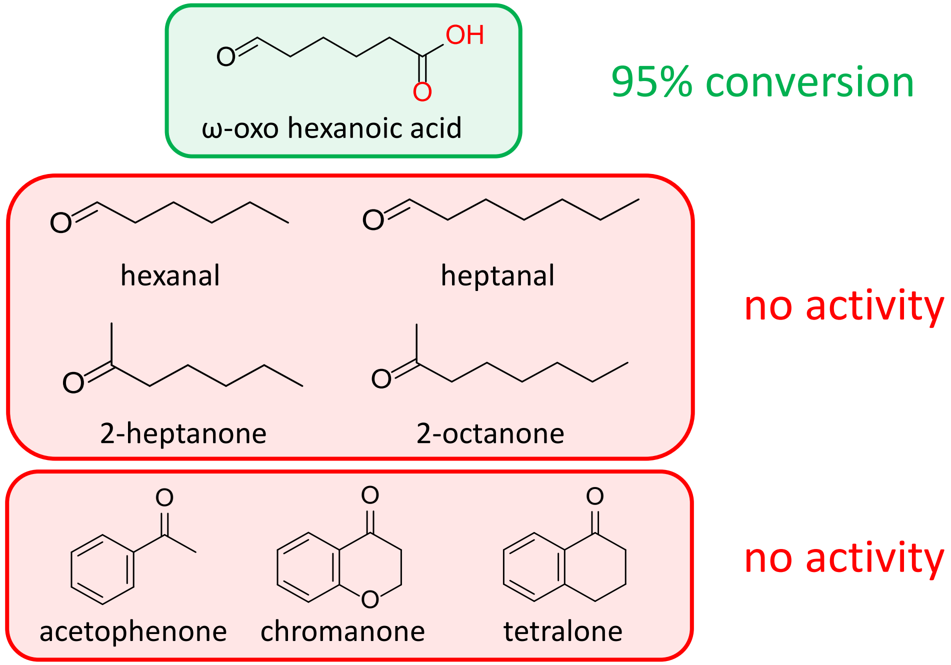
LysEDH catalyses the formally irreversible oxidative deamination of the ε-amino group of L-Lysine

AN ALTERNATIVE STRATEGY TO ENGINEER AmDHs: ε-DEAMINATING L-LYSINE DEHYDROGENASE

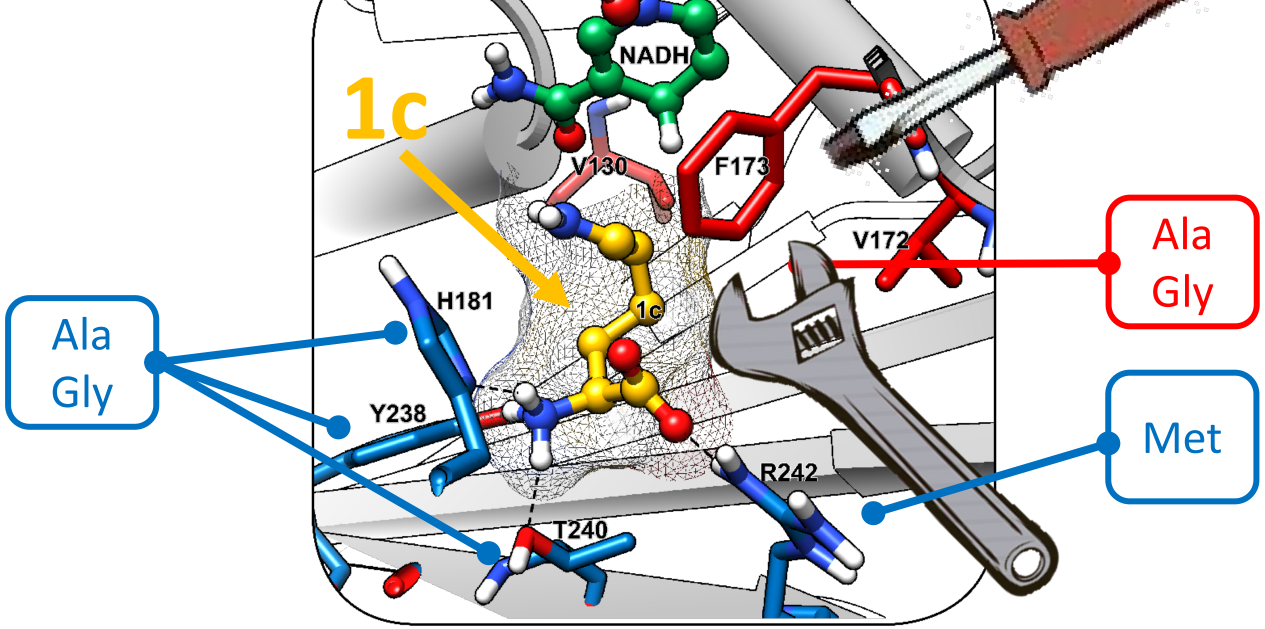
Geobacillus stearothermophilus ε-lysine dehydrogenase[1] (LysEDH) catalyses the irreversible oxidative deamination of the ε-amino group of L-Lysine



Initial activity test (reductive amination)

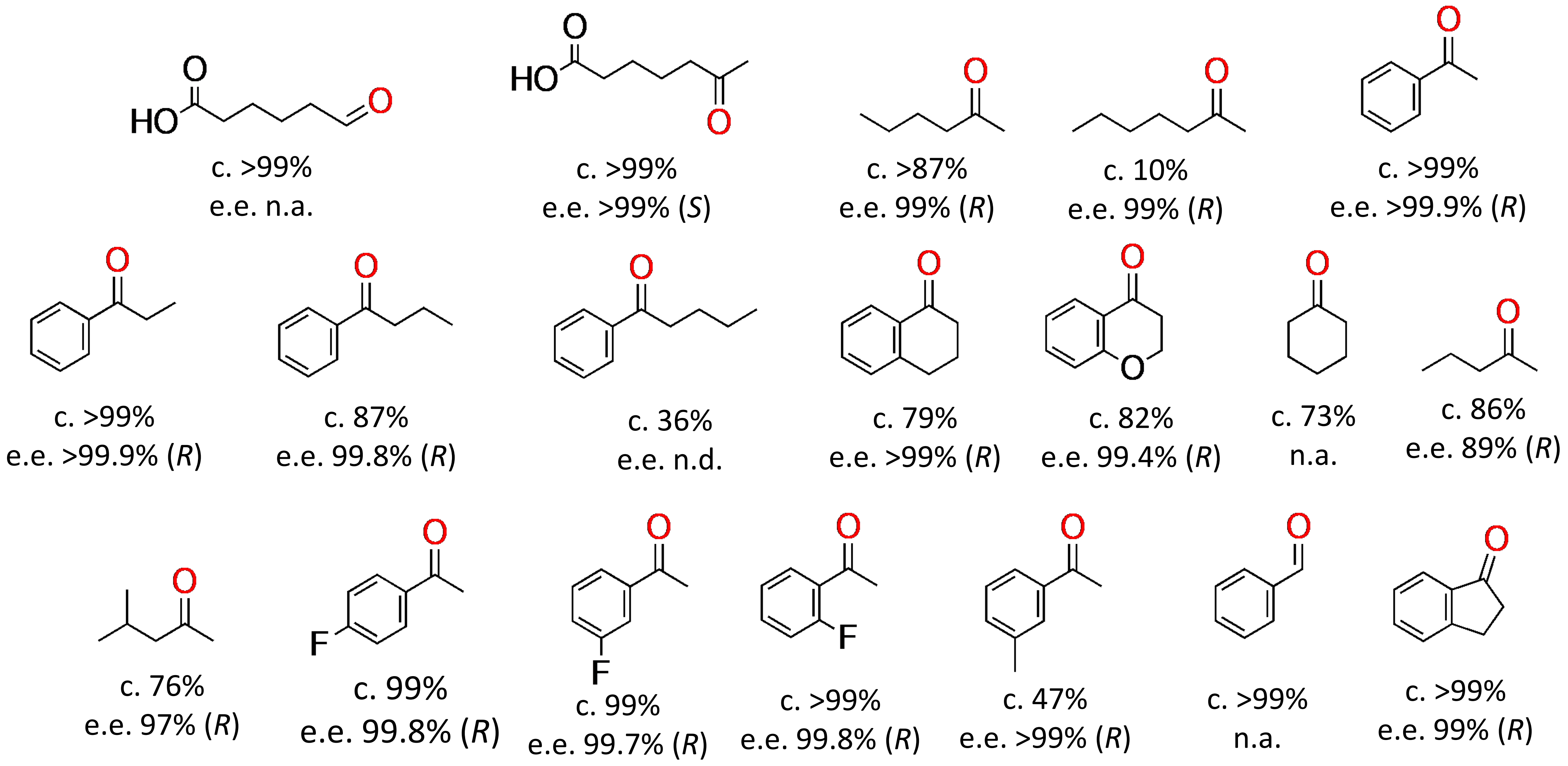


Homology model of LysEDH containing its natural substrate 1c



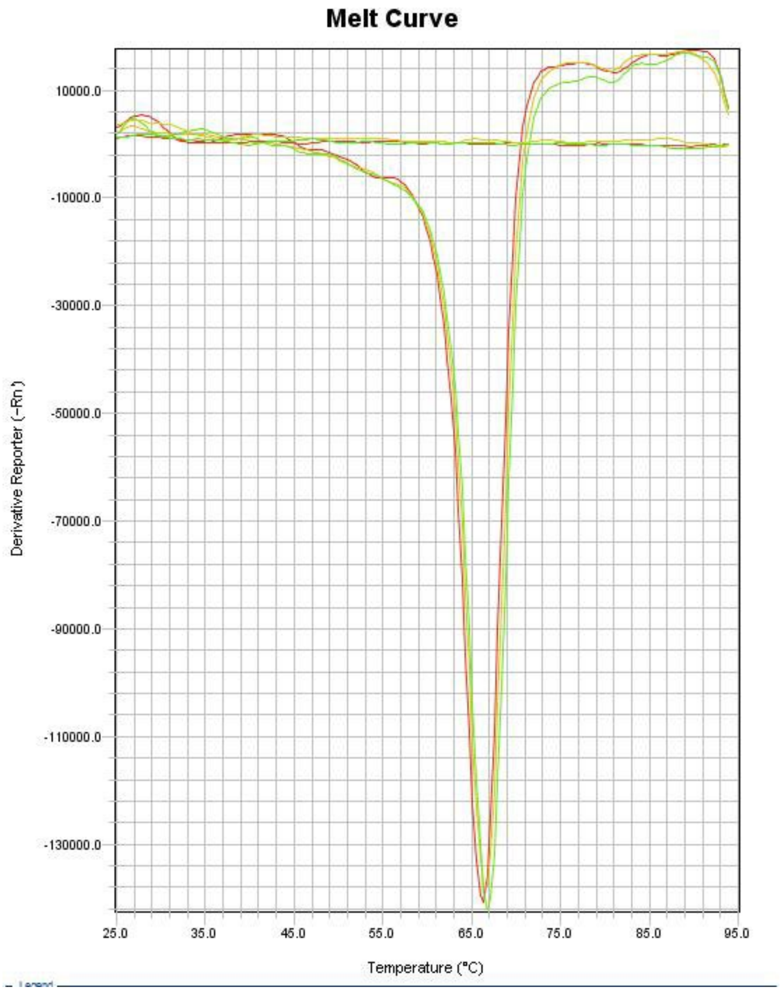
V. Tseliou, T. Knaus, M. F. Masman, M. L. Corrado, F. G. Mutti, *Nat. Commun.* **2019**, 10, 3717.

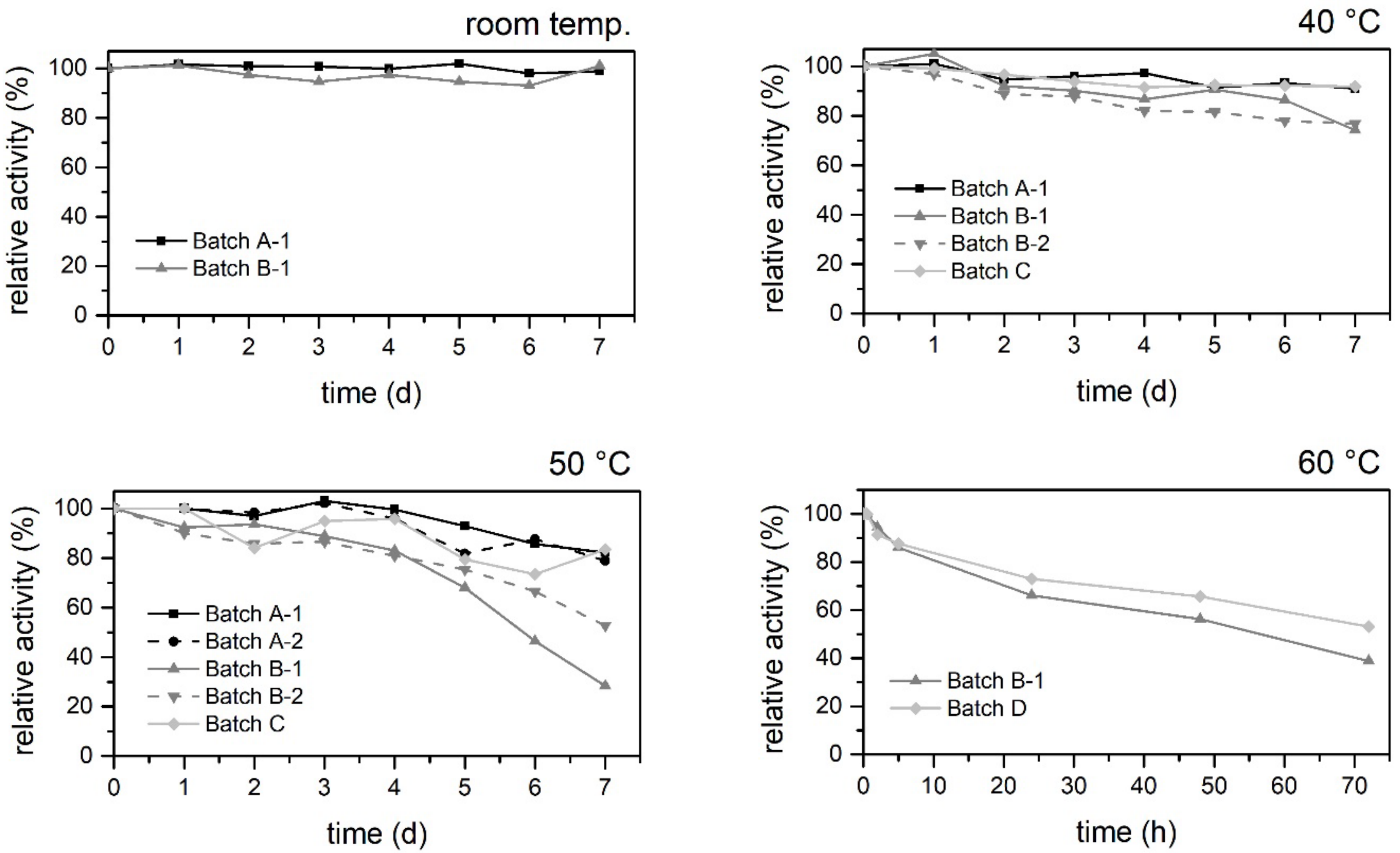
Testing of LE-AmDH-v1



V. Tseliou, T. Knaus, M. F. Masman, M. L. Corrado, F. G. Mutti, *Nat. Commun.* **2019**, 10, 3717.
F. Mutti, V. Tseliou, T. Knaus, M. F. Masman, WO 2020165417, 2020.

THERMAL STABILITY OF LE-AmDH-v1

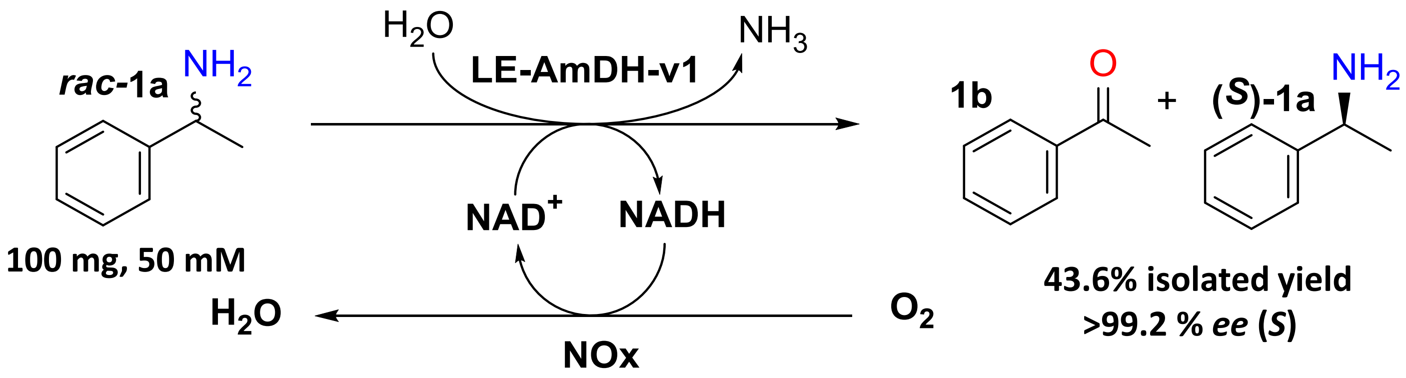


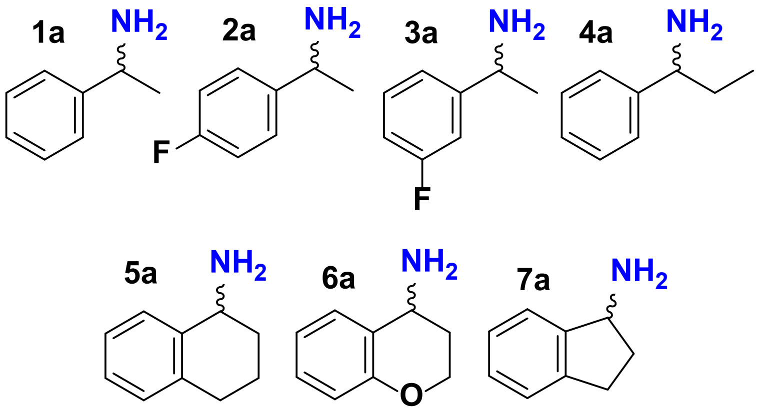


Tm = 69 °C

V. Tseliou, T. Knaus, M. F. Masman, M. L. Corrado, F. G. Mutti, *Nature Commun.* **2019**, 10, 3717.
F. Mutti, V. Tseliou, T. Knaus, M. F. Masman, WO 2020165417, 2020.

Kinetic Resolution with LE-AmDH-v1



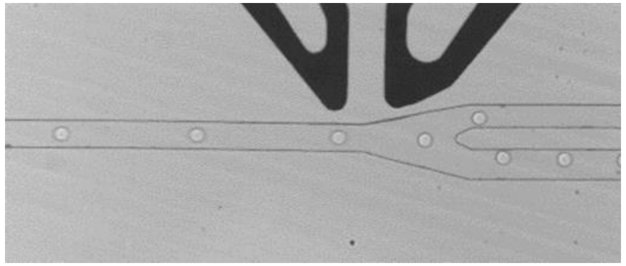


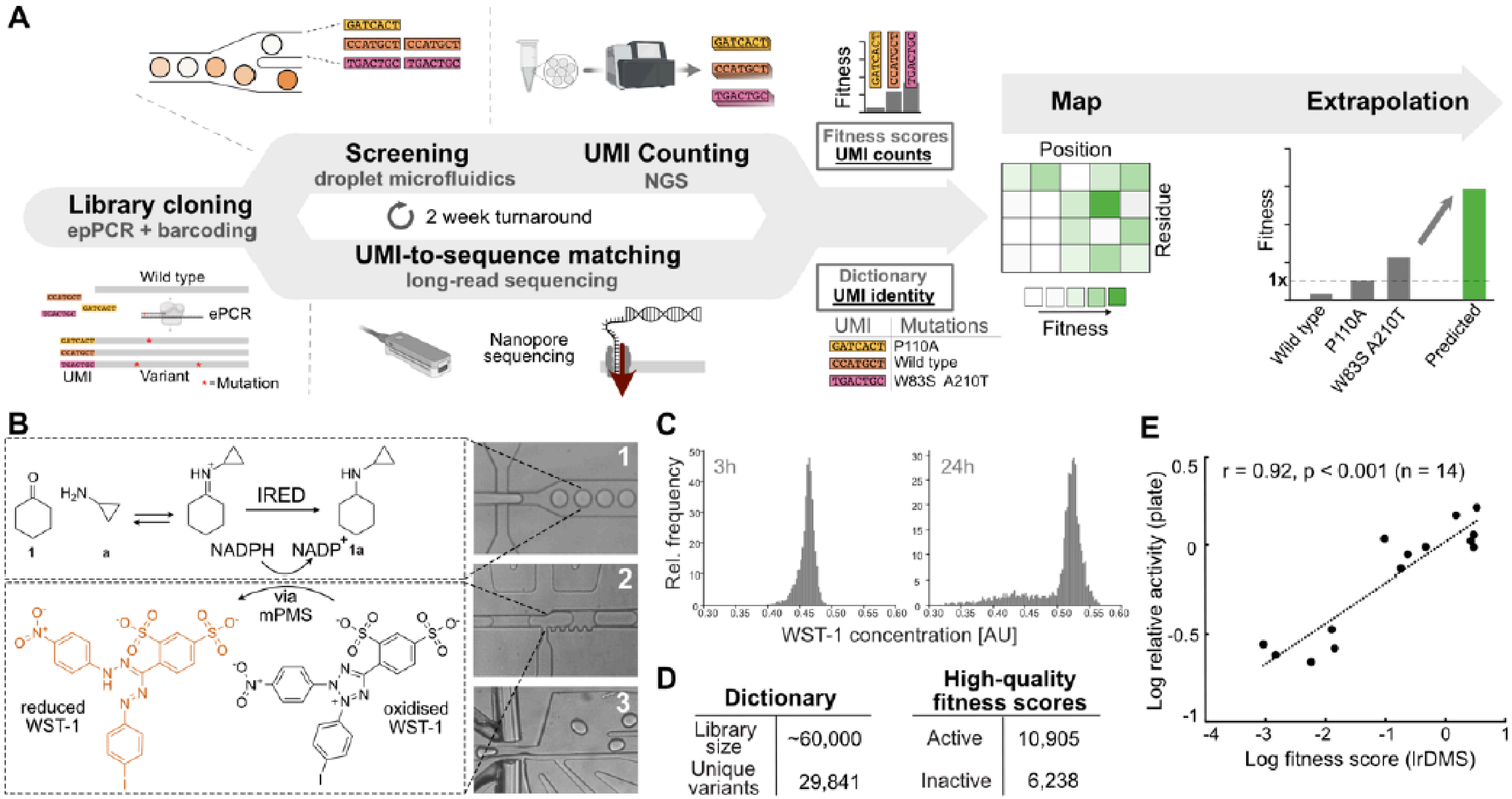
Substrate	Time (h)	Conv. (%)[a]	ee
1a	24	49.8	>99.3 % (S)
2a	48	49.7	>99.2 % (S)
3a	48	46.1	95 % (S)
4a	24	50.3	>99.4 % (S)
5a	24	49.4	>99.5 % (S)
6a	24	49.8	>99.3 % (S)
7a	24	50.4	>99.6 % (S)

V. Tseliou, T. Knaus, J. Vilím, M. F. Masman, F. G. Mutti, *ChemCatChem* **2020**, 12, 2184-2188.

AI-ASSISTED ENGINEERING AND MICRODROPLET SCREENING FOR RAPID PROFILE OF BIOCATALYST

Exemplified for engineering of imine reductases (IREDs)

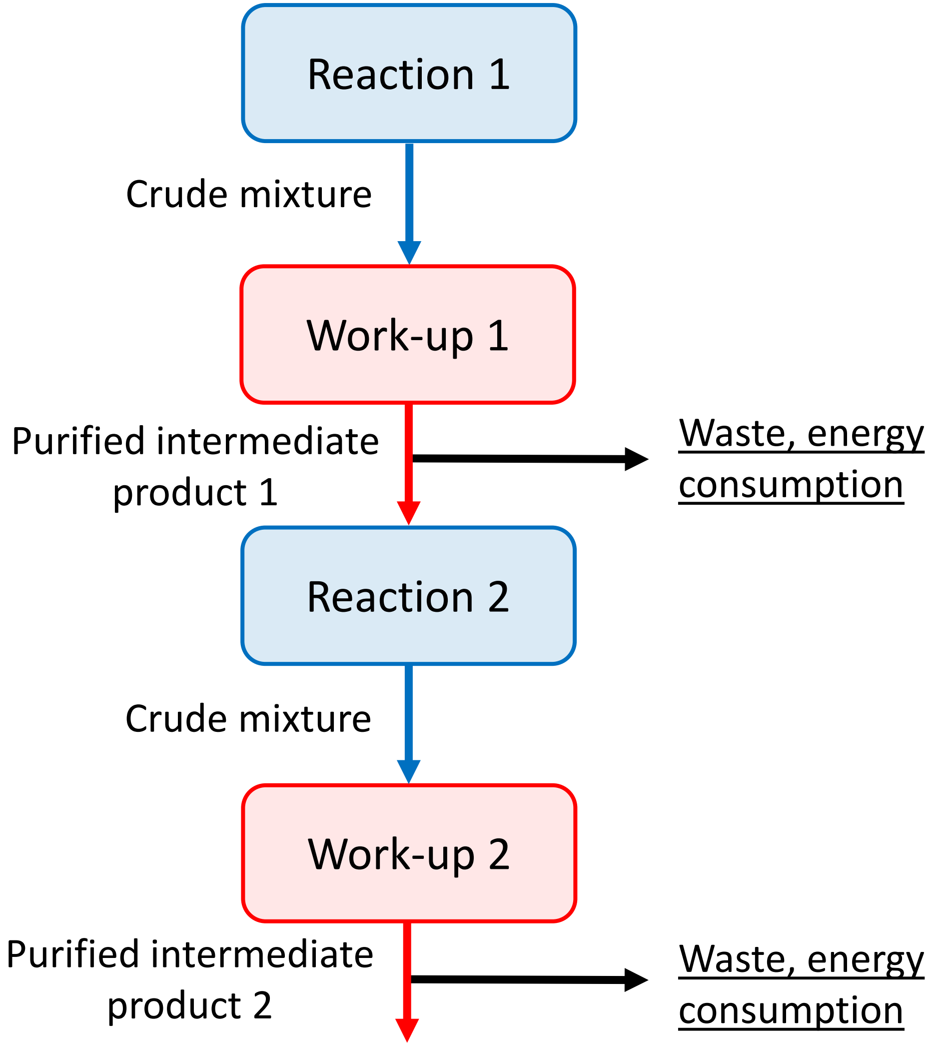




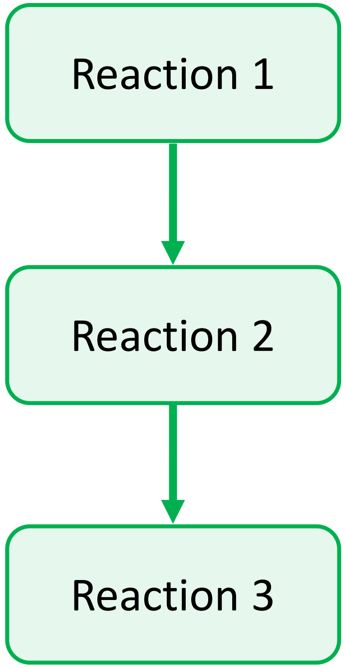
M. Gantz, S. V. Mathis, F. E. H. Nintzel, P. J. Zurek, T. Knaus, E. Patel, D. Boros, F.-M. Weberling, M. R. A. Kenneth, O. J. Klein, E. J. Medcalf, J. Moss, M. Herger, T. S. Kaminski, F. G. Mutti, P. Lio, F. Hollfelder, *bioRxiv* **2024**, DOI: 10.1101/2024.1104.1108.588565. [University of Cambridge, University of Amsterdam]

HIMSBiocat

Traditional concept of chemical synthesis



Cascade chemistry

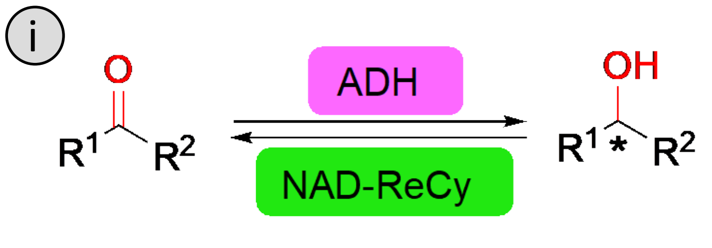


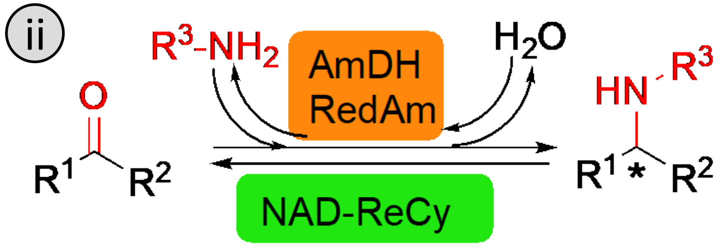
Reduced waste generation, save time and money

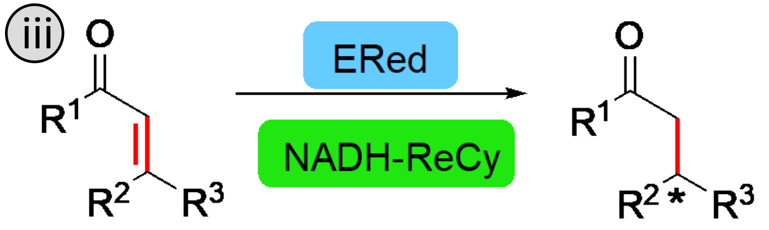
Requirements:

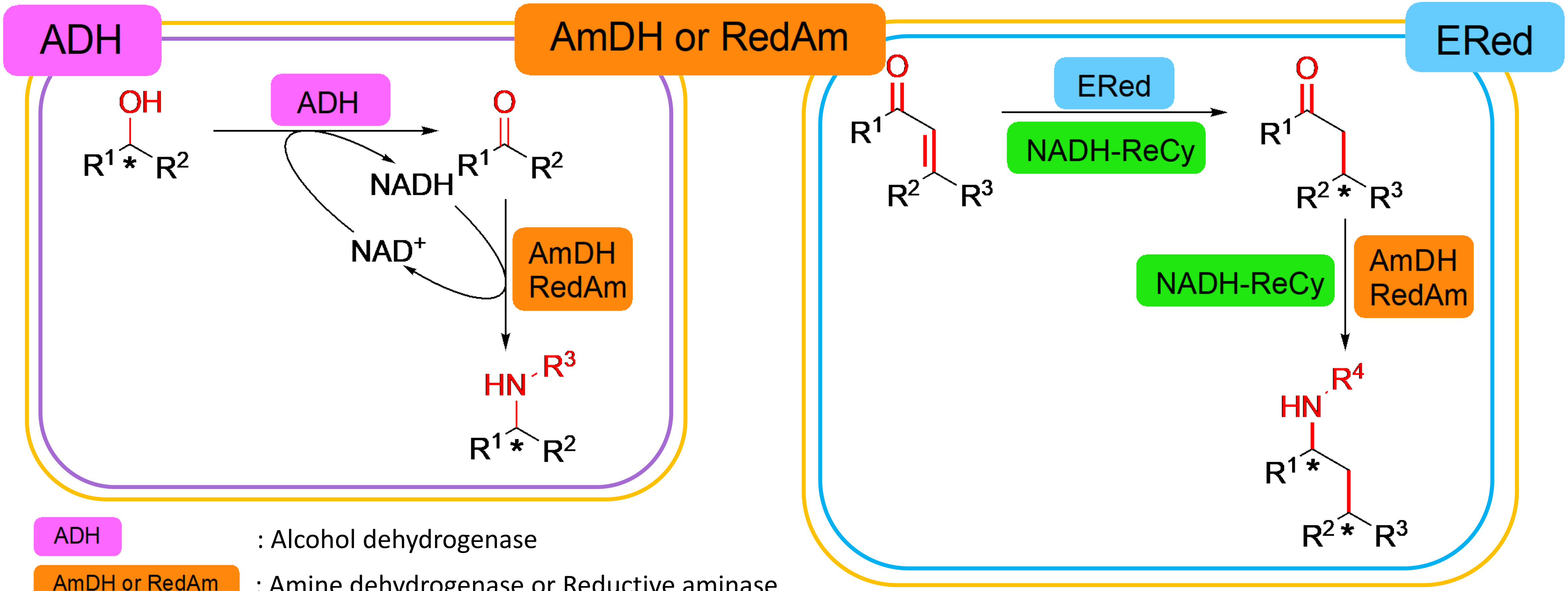
1. **Compatible catalysts**
2. **Very selective catalysts**

EXAMPLES OF BIOCATALYTIC CASCADES TO AMINES AND AMINO ALCOHOLS









ADH : Alcohol dehydrogenase

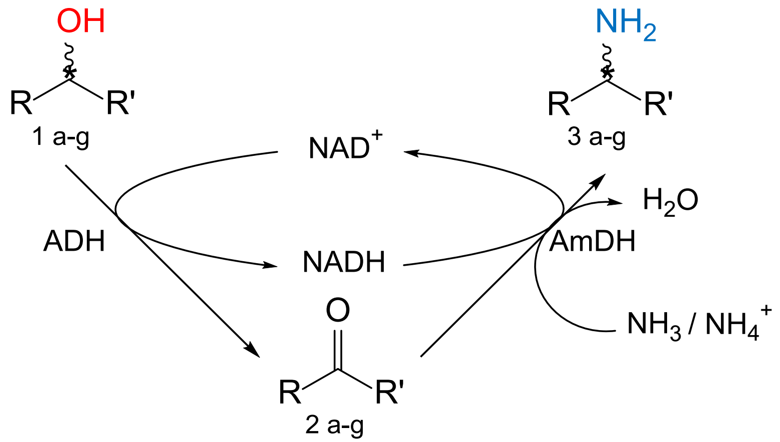
AmDH or RedAm : Amine dehydrogenase or Reductive aminase

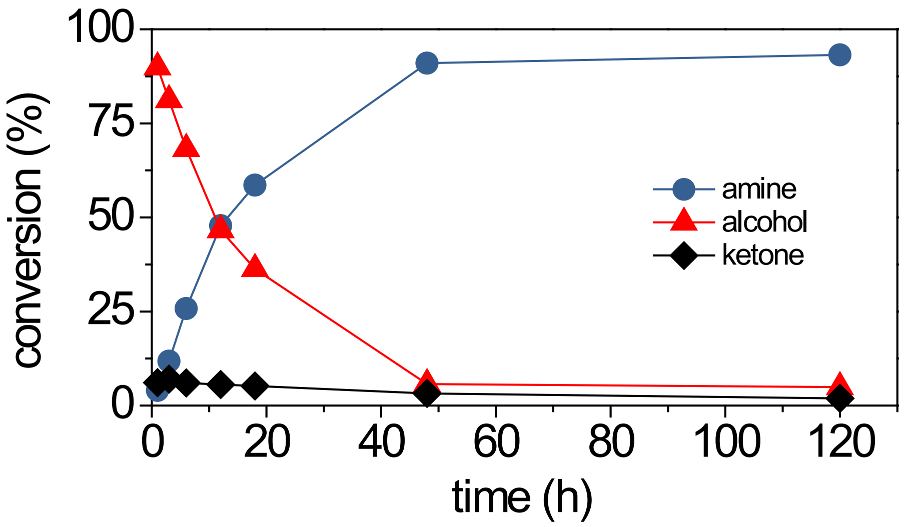
ERed : Ene-reductase

Asymmetric hydride-borrowing bio-amination of alcohols

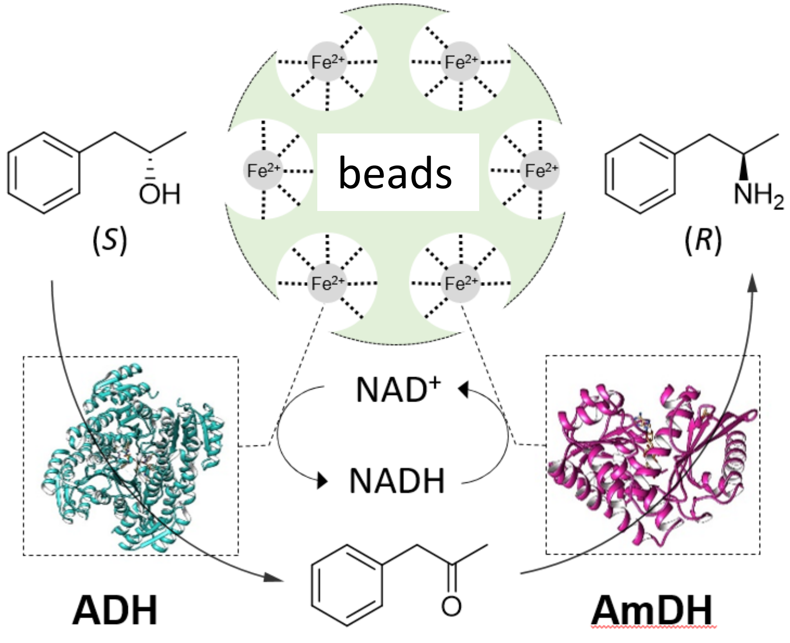
Elevated atom efficiency, chemo- and stereoselectivity. Enantiomeric excess >99% (*R*).

1st generation: isolated enzymes





2nd generation: co-immobilised dehydrogenases

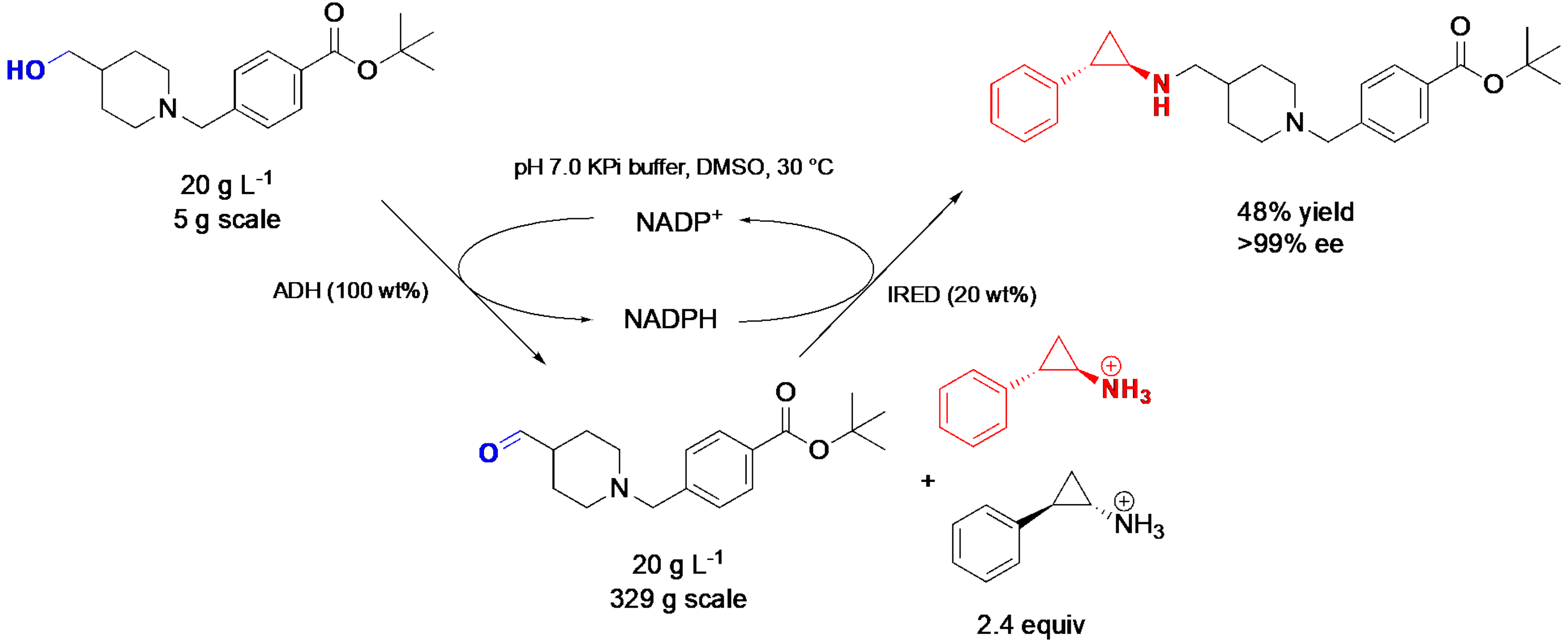


For each cycle:

- TON ADH = ca. 2700
- TON AmDH = ca. 900

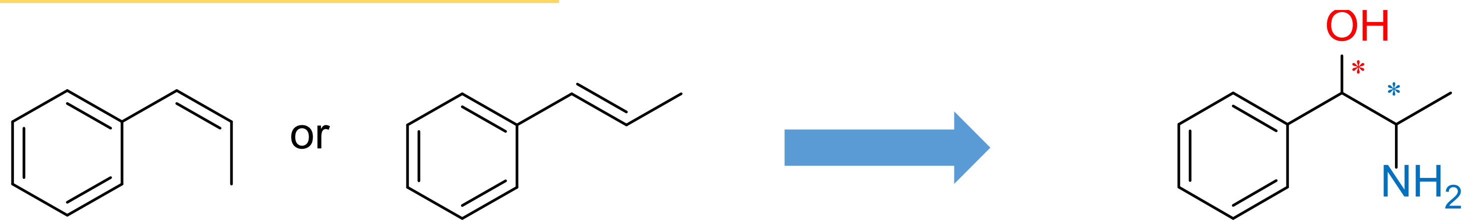
F. G. Mutti*, T. Knaus, N. S. Scrutton, M. Breuer, N. J. Turner*, *Science* **2015**, *349*, 1525 – 1529.
W. Böhmer, T. Knaus, F. G. Mutti, *ChemCatChem* **2018**, 10, 731-735.

Example for the chiral synthesis of LSD1 inhibitor GSK2879552 (by GSK)

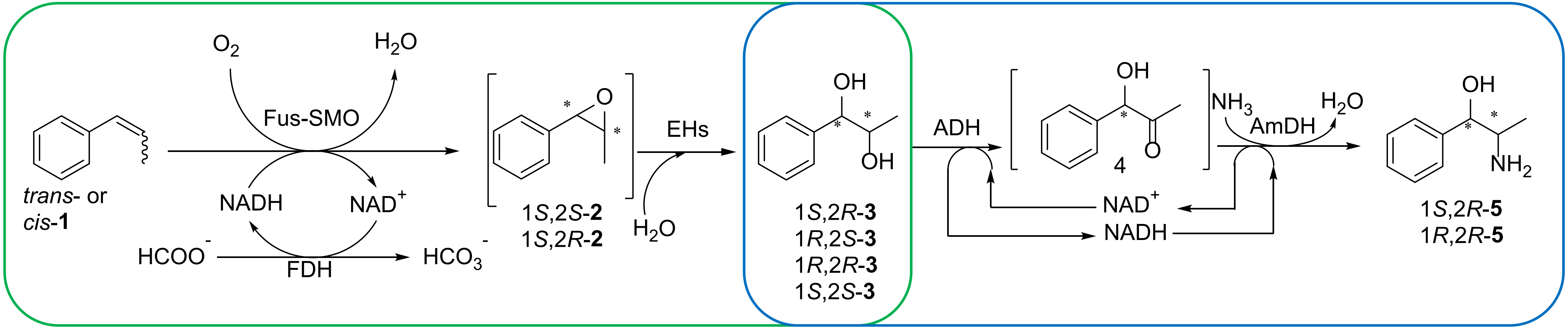


M. Schober, C. MacDermaid, A. A. Ollis, S. Chang, D. Khan, J. Hosford, J. Latham, L. A. F. Ihnken, M. J. B. Brown, D. Fuerst, M. J. Sanganee, G.-D. Roiban, *Nat. Catal.* **2019**, 2, 909-915.

REGIO- AND STEREOSELECTIVE MULTI-ENZYMATIC AMINOHYDROXYLATION OF β-METHYLSTYRENE



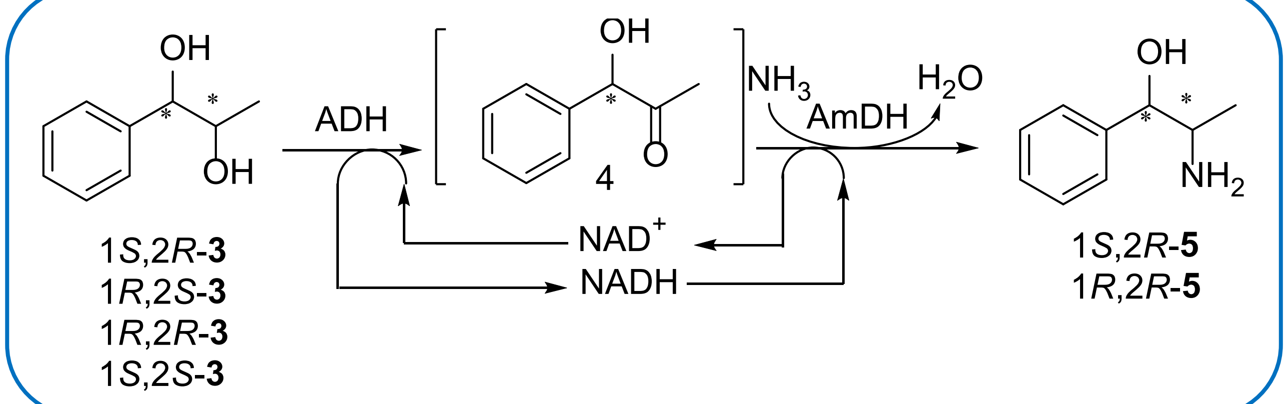
Cascade 1 | Cascade 2



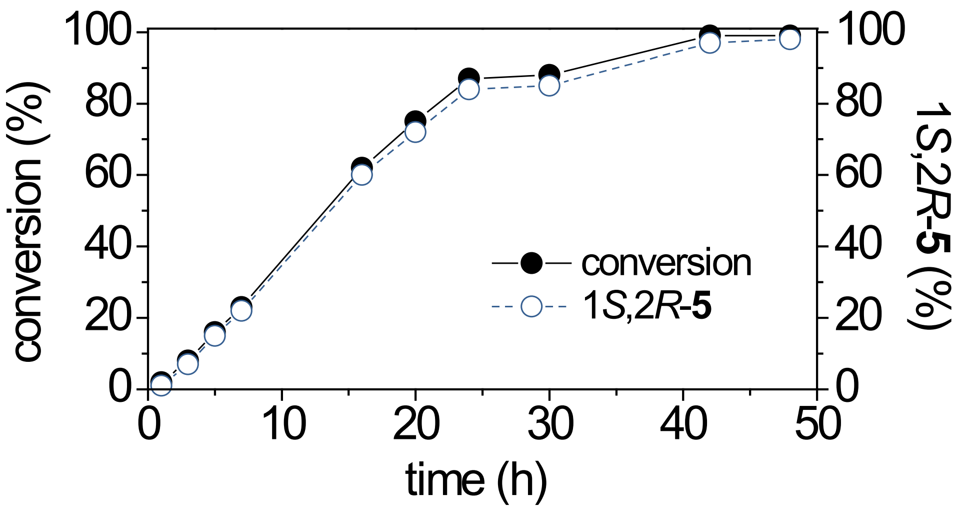
Substrate	Step 1 Isolated Yield [%]	Step 2 Isolated Yield [%]	Combined Yield [%]	*er* [%]	*dr* [%]
trans-**1**	85	74	63 (1*S*,2*R*-**5**)	>99.5 : <0.5	98 : 2[a]
cis-**1**	79	74	59 (1*R*,2*R*-**5**)	>99.5: <0.5	>99.5: <0.5

M. L. Corrado, T. Knaus, F. G. Mutti *Green Chem.* **2019**, *21*, 6246-6251.
M. L. Corrado, T. Knaus, F. G. Mutti, *ChemBioChem* **2018**, *19*, 679-686.

REGIO- AND STEREOSELECTIVE MULTI-ENZYMATIC AMINOHYDROXYLATION OF β-METHYLSTYRENE

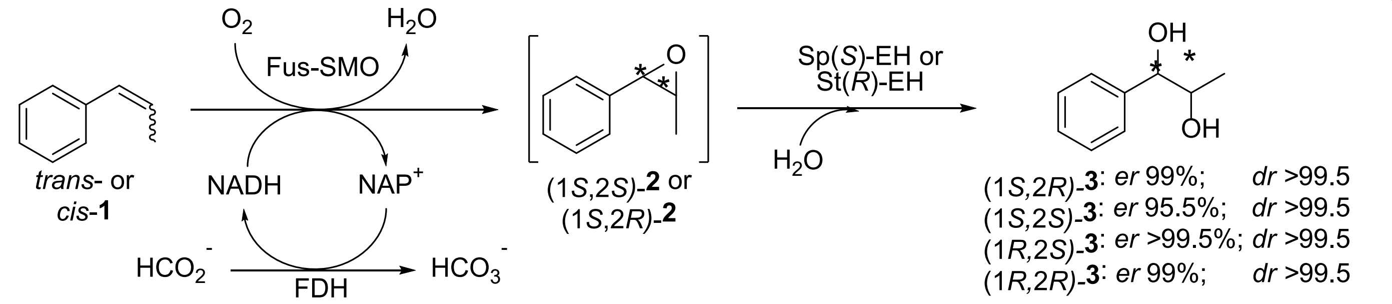


Progress of representative alcohol amination (from 1*S*,2*S*-**3** to 1*S*,2*R*-**5**) > 100 mg scale

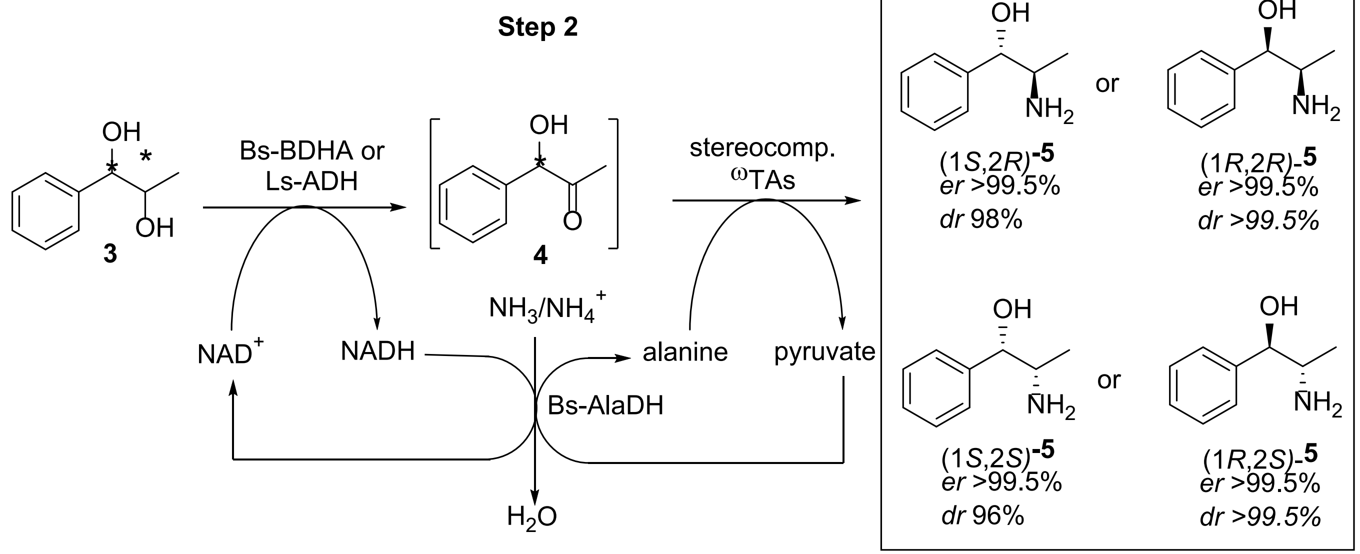


M. L. Corrado, T. Knaus, F. G. Mutti *Green Chem.* **2019**, *21*, 6246-6251.
M. L. Corrado, T. Knaus, F. G. Mutti, *ChemBioChem* **2018**, *19*, 679-686.

REGIO- AND STEREOSELECTIVE MULTI-ENZYMATIC AMINOHYDROXYLATION OF β-METHYLSTYRENE: AN ALTERNATIVE NETWORK



Cascade 1



Cascade 2

All four possible stereoisomers were obtained

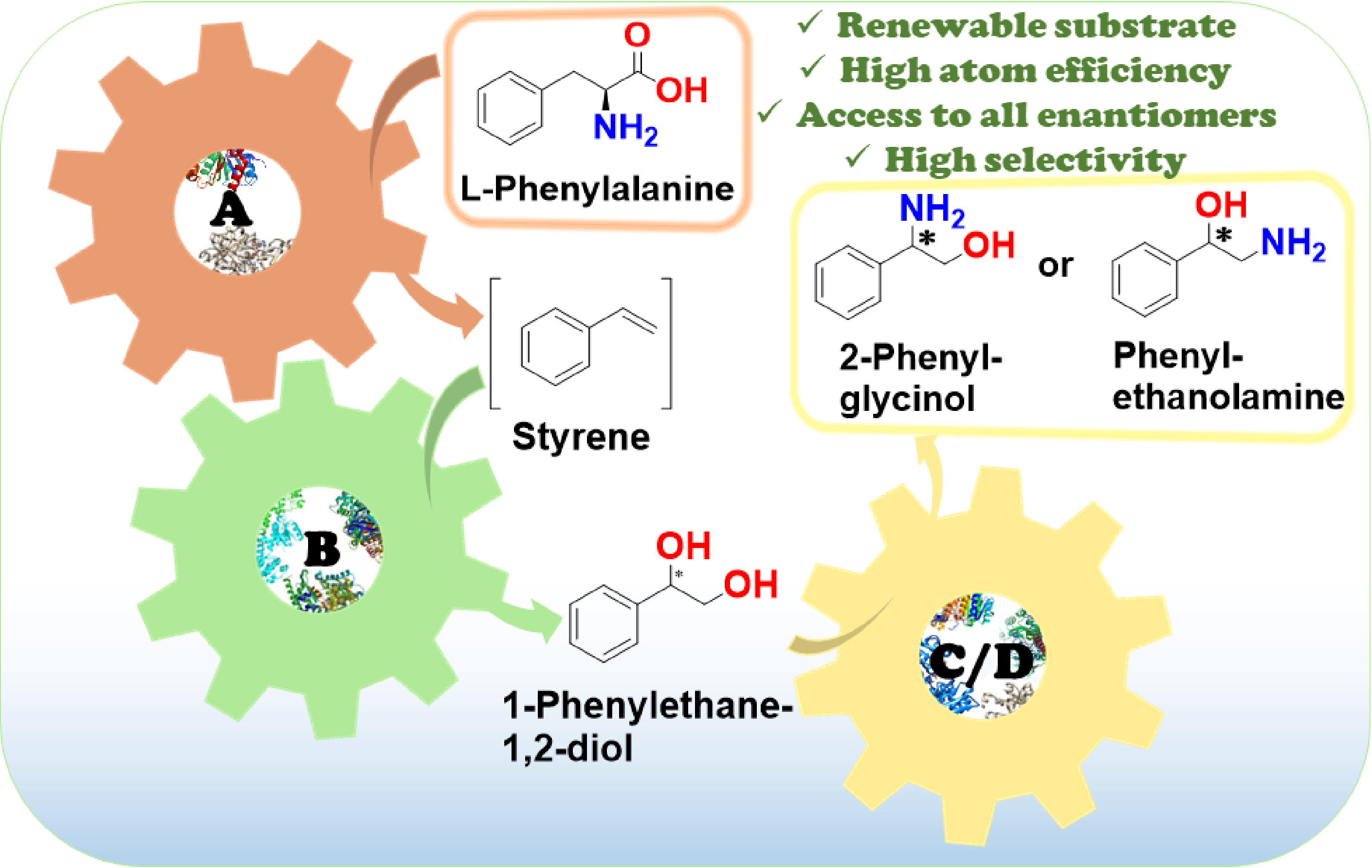
Substrate	Step 1, Yield [%]	Step 2, Yield [%]	Combined, Yield [%]	*er* [%]	*dr* [%]
trans-**1**	86	83	71 (1*S*,2*S*-**5**)	>99.5 : <0.5	97 : 3
cis-**1**	70	75	53 (1*R*,2*S*-**5**)	>99.5: <0.5	>99.5: <0.5

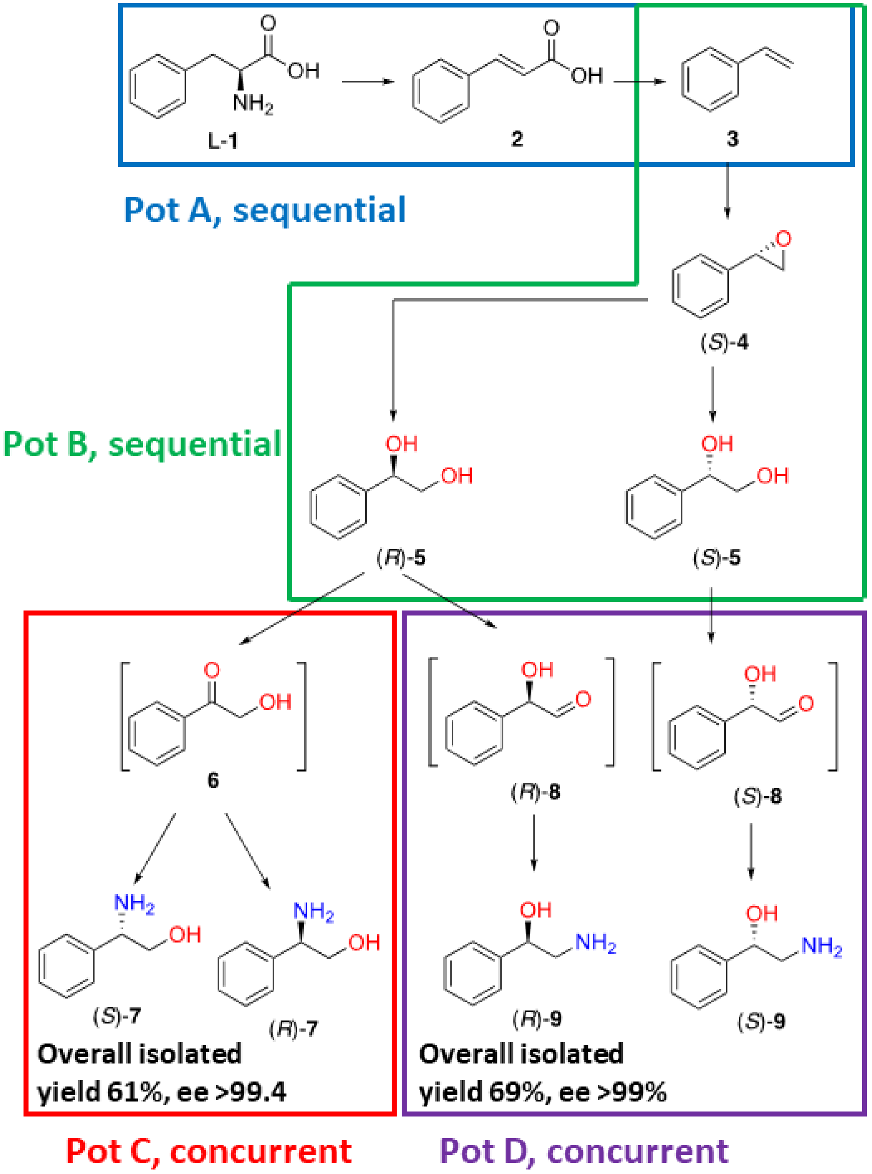
M. L. Corrado, T. Knaus, F. G. Mutti, *ChemBioChem* **2021**, *22*, 2345–2350.

HIGH YIELD SYNTHESIS OF 1,2-AMINO ALCOHOLS FROM L-PHENYLALANINE

HIMSBiocat

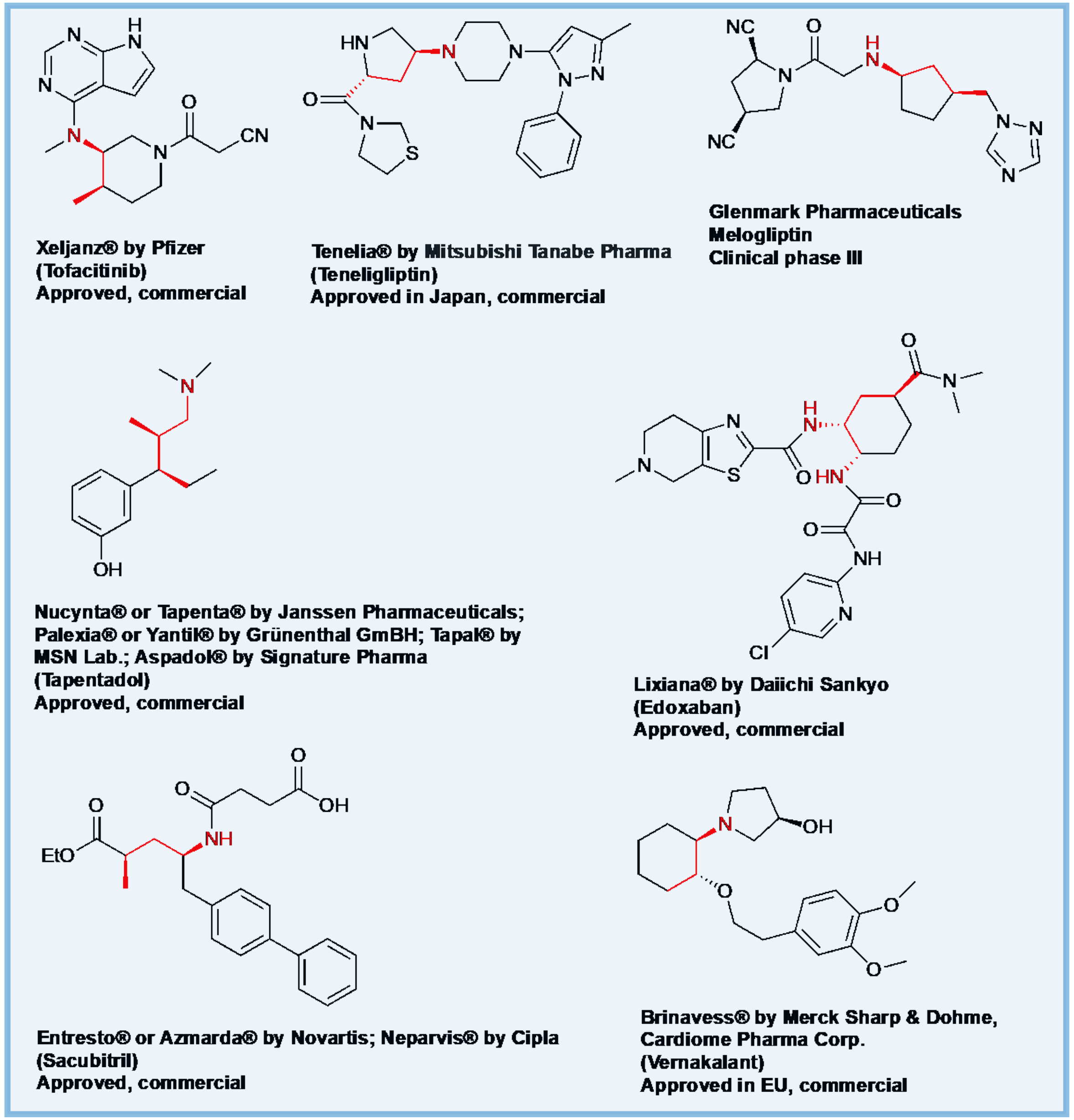
Linear and divergent enzymatic cascades

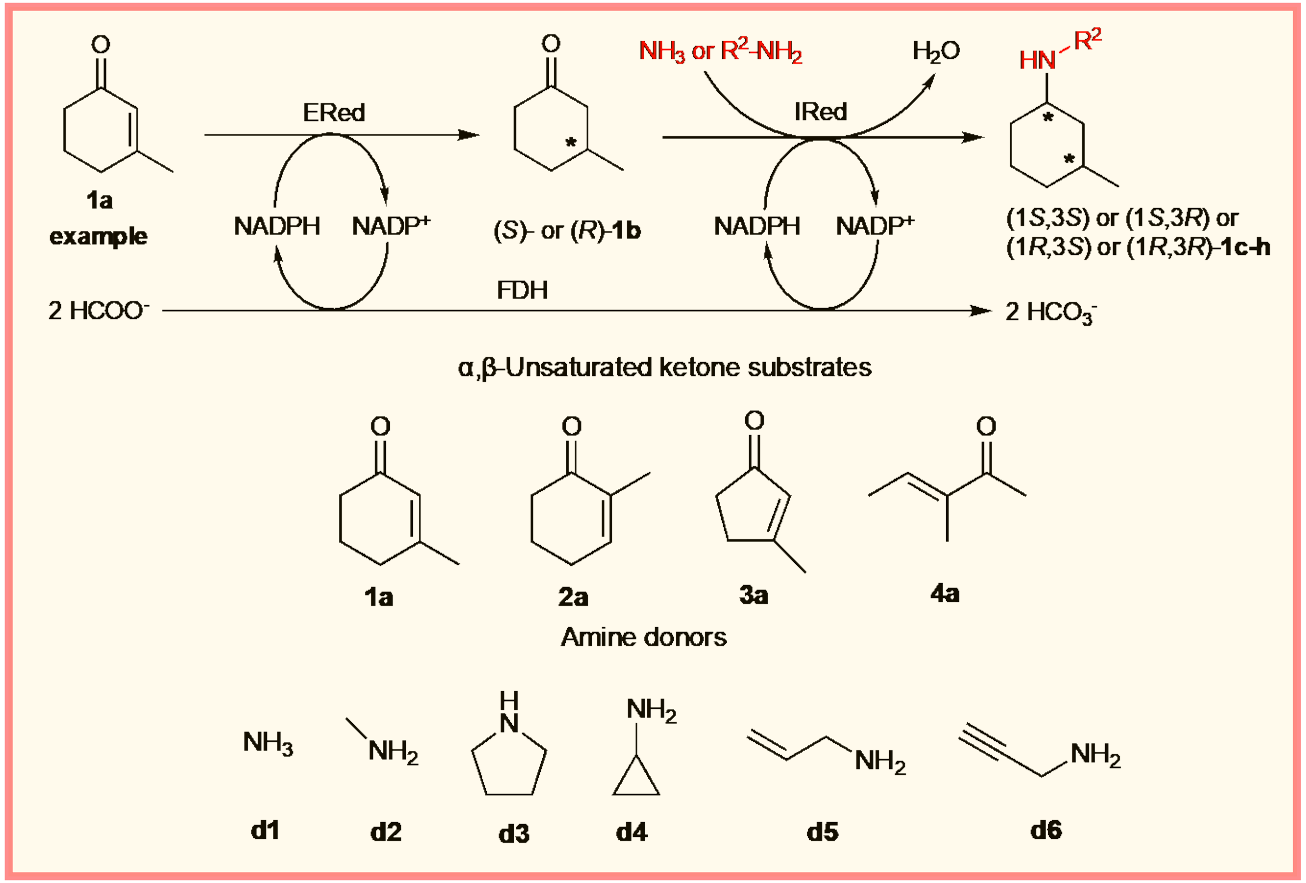




M. L. Corrado, T. Knaus, U. Schwaneberg, F. G. Mutti, *Org. Process Res. Dev.* **2022**, 26, 2085-2095.

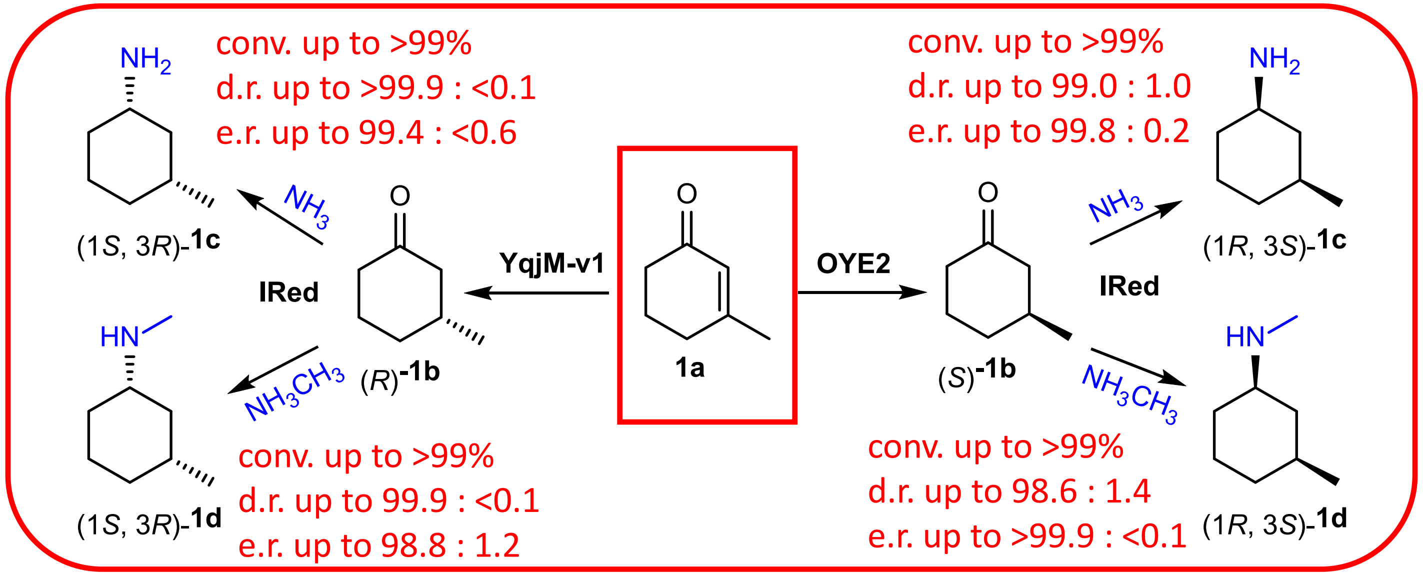
ERED-IRED CASCADES: ACCESS TO PRIMARY, SECONDARY AND TERTIARY AMINES WITH TWO STEREOCENTERS

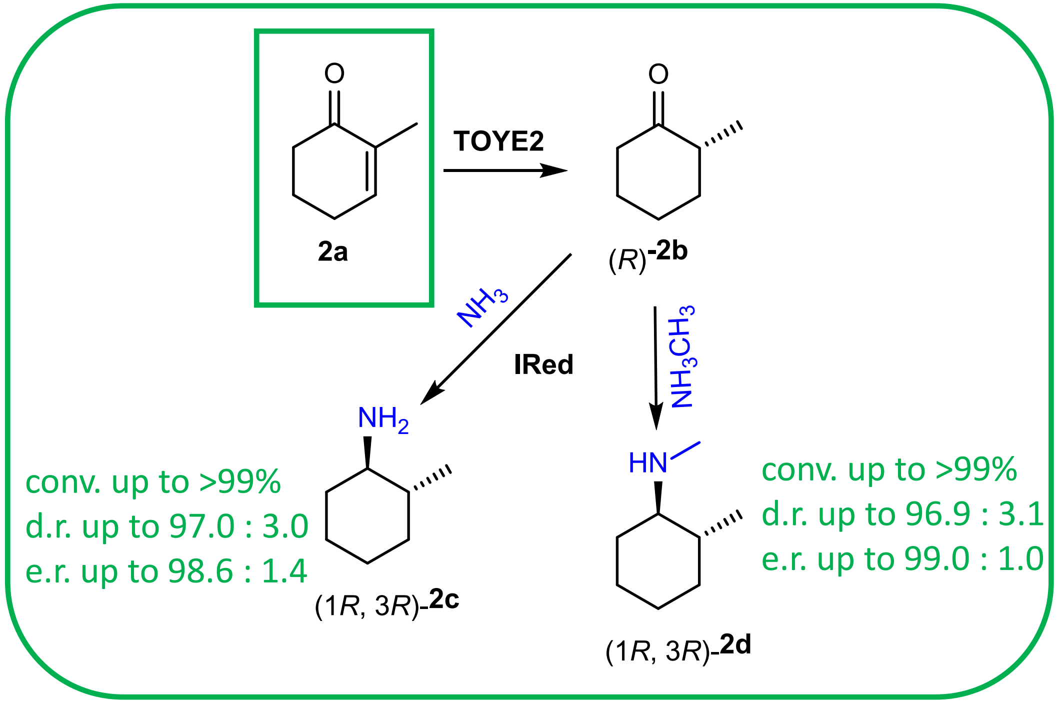


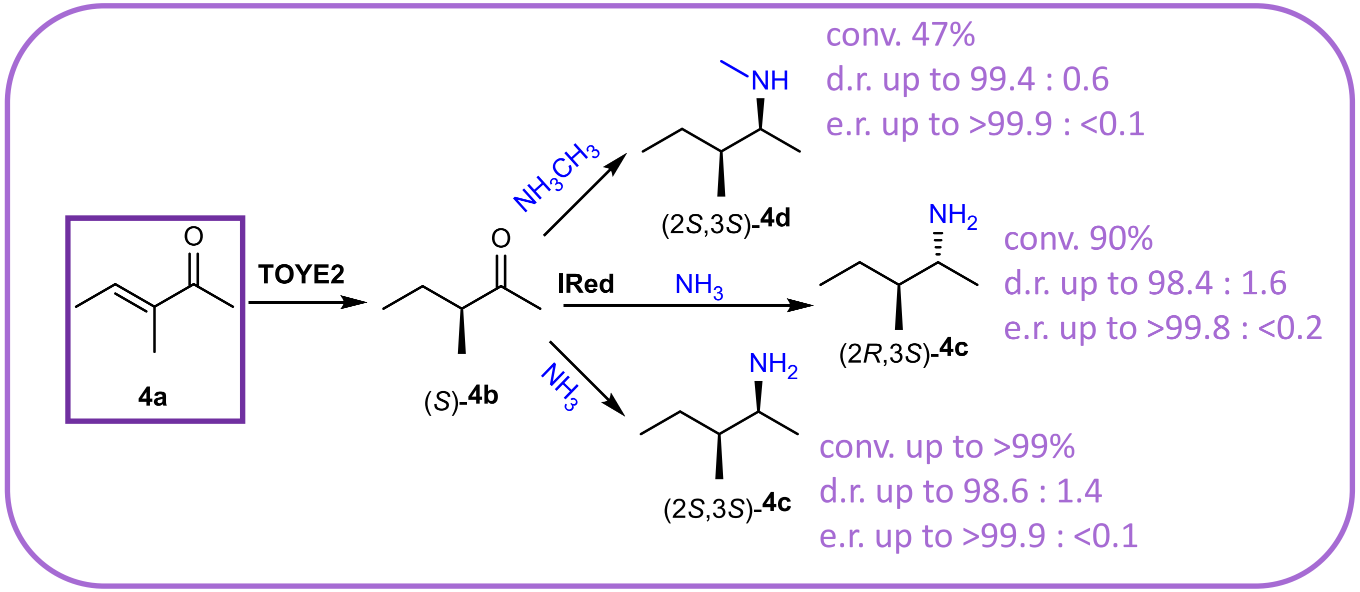


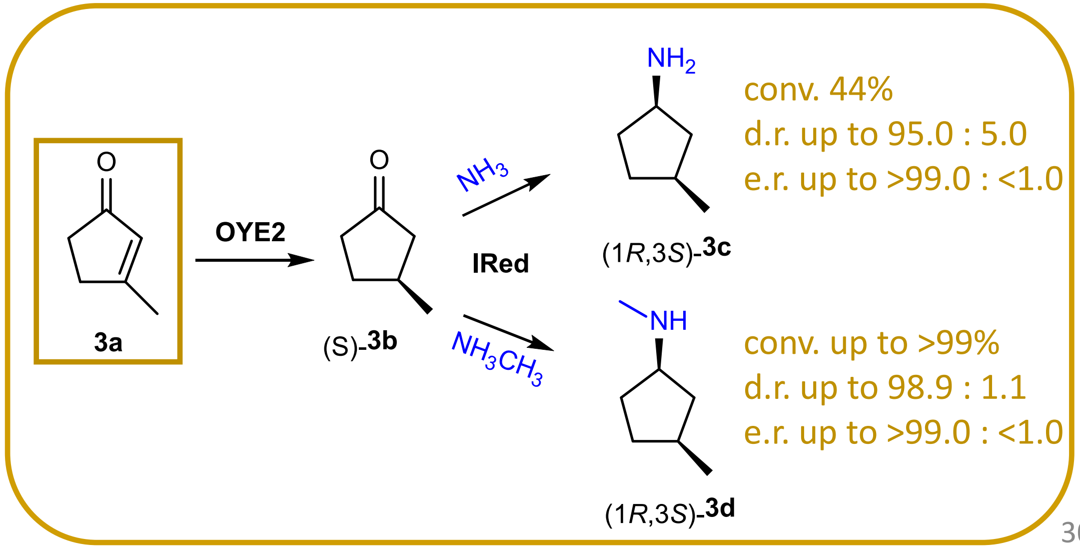
T. Knaus, M. L. Corrado, F. G. Mutti, *ACS Catal.* **2022**, *12*, 14459–14475.

ERED-IRED CASCADES: ACCESS TO PRIMARY, SECONDARY AND TERTIARY AMINES WITH TWO STEREOCENTERS



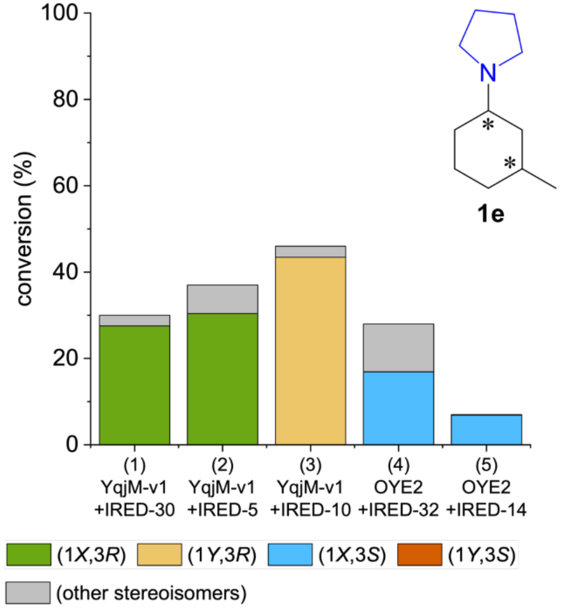


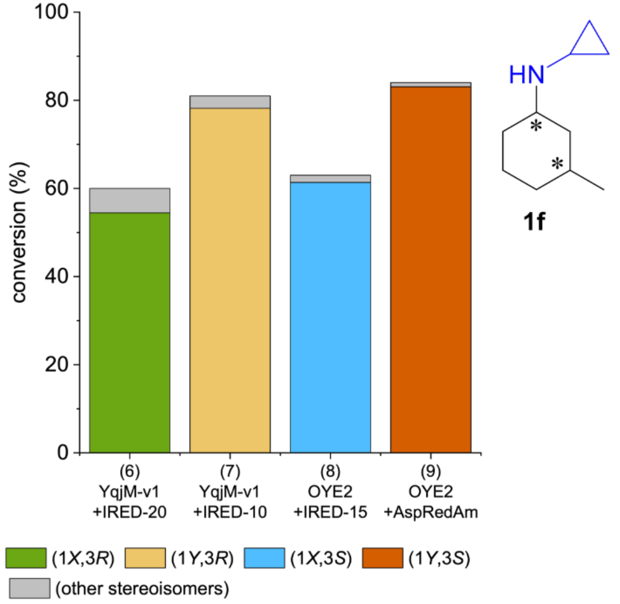


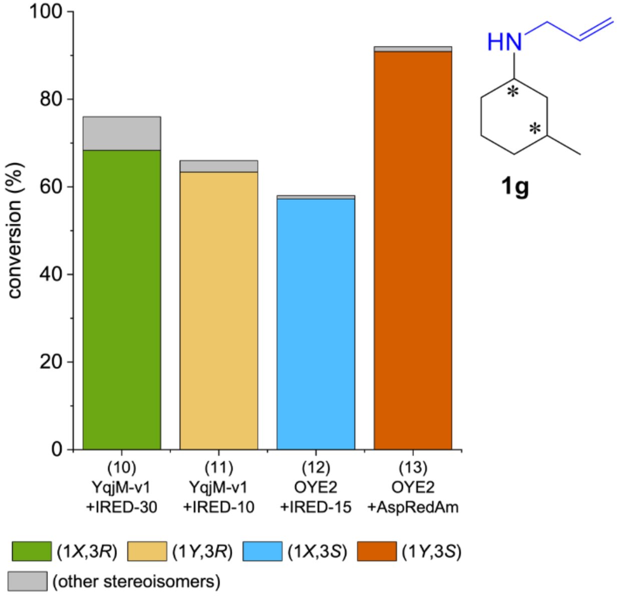


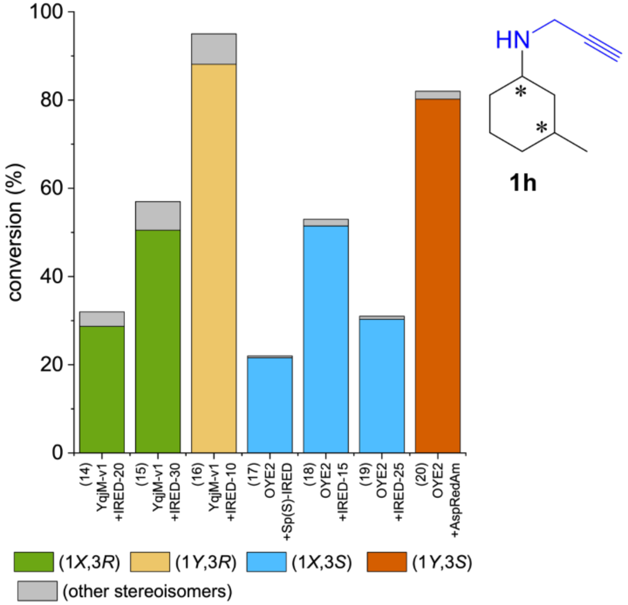
T. Knaus, M. L. Corrado, F. G. Mutti, *ACS Catal.* **2022**, *12*, 14459–14475.

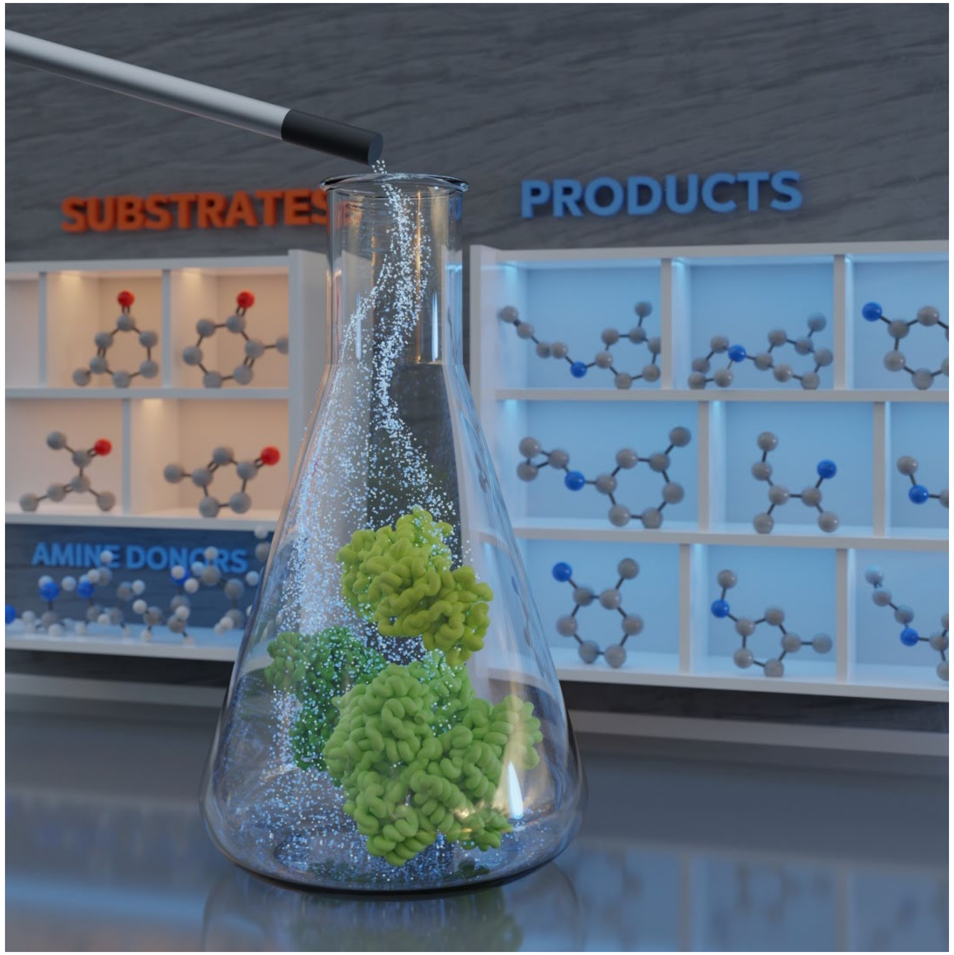
ERED-IRED CASCADES: ACCESS TO PRIMARY, SECONDARY AND TERTIARY AMINES WITH TWO STEREOCENTERS









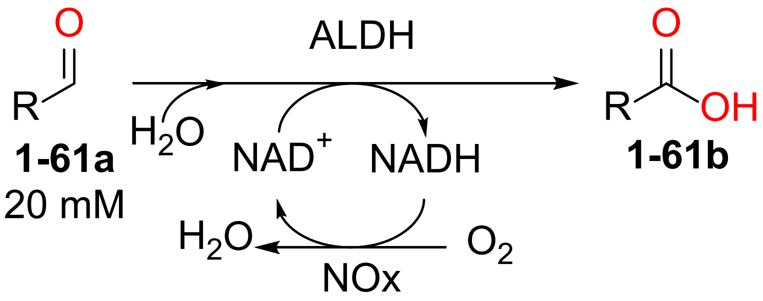


T. Knaus, M. L. Corrado, F. G. Mutti, *ACS Catal.* **2022**, *12*, 14459–14475.

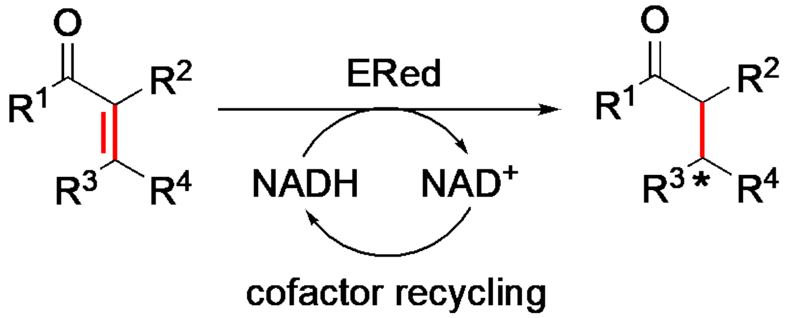
ANOTHER HYDRIDE-BORROWING CASCADE: COMBINING ALDEHYDE DEHYDROGENASES AND ENE-REDUCTASES

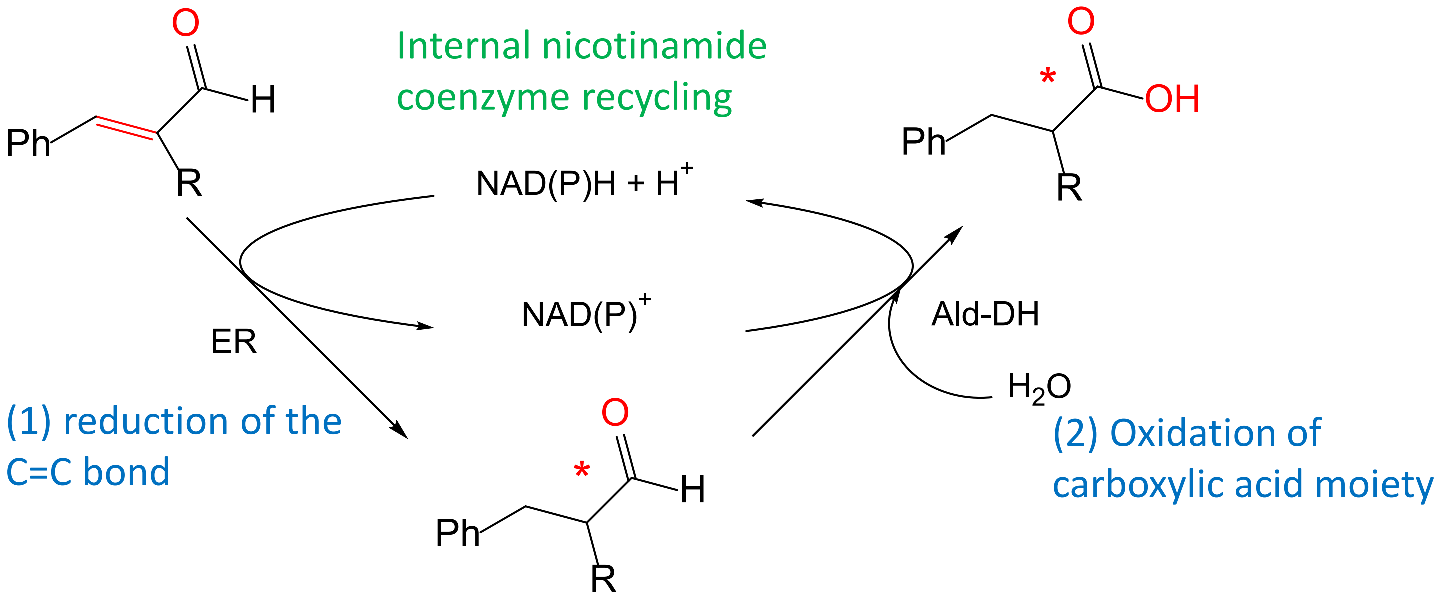
HIMSBiocat

Aldehyde dehydrogenases



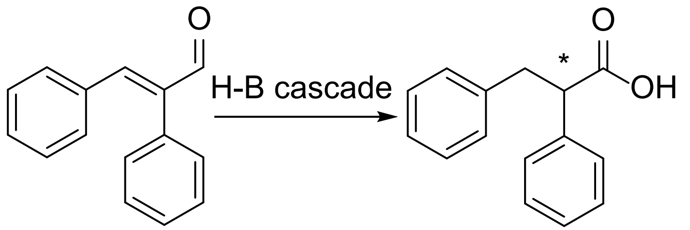
Ene-reductases





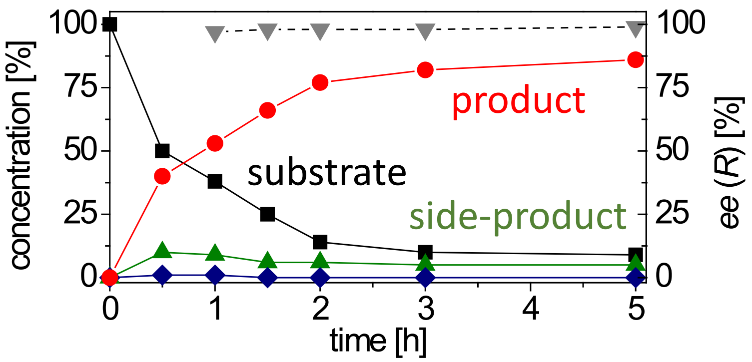
T. Knaus et al. *Org. Biomol. Chem.* **2017**, 15, 8313-8325

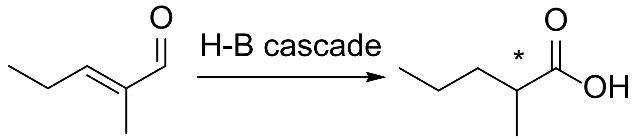
HYDRIDE-BORROWING CASCADE REACTION TO CHIRAL α-SUBSTITUTED CARBOXYLIC ACIDS



95% chemoselectivity

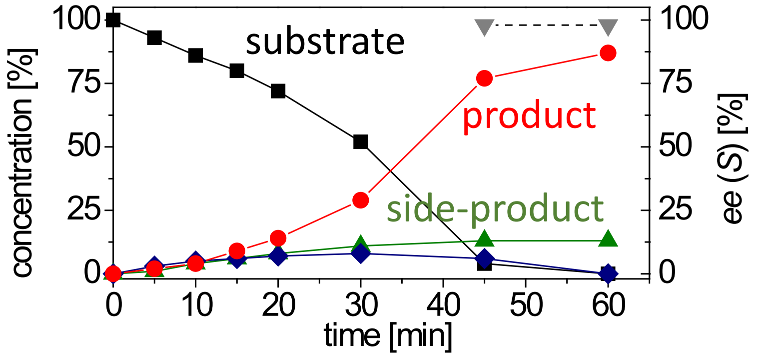
>98% *ee* (*R*)

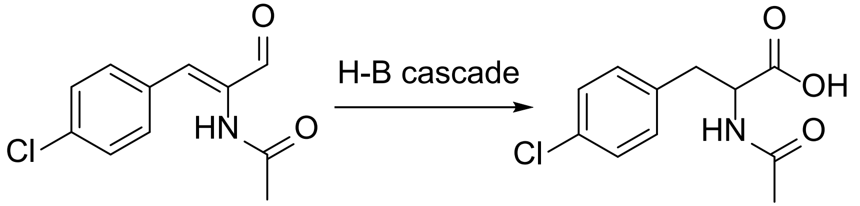




92% chemoselectivity

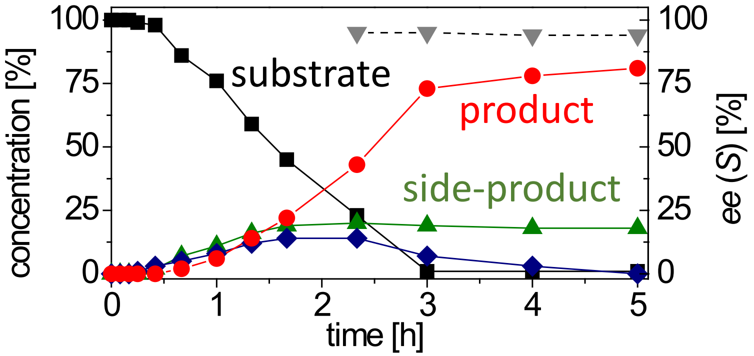
>98% *ee* (*S*)

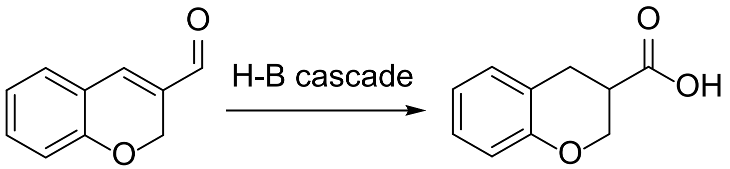




81% chemoselectivity

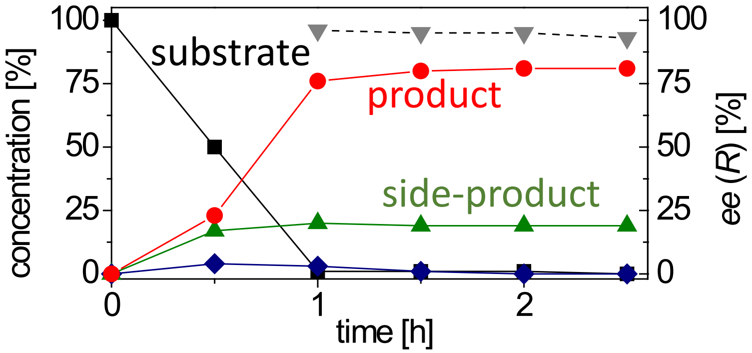
>95% *ee* (*S*)





82% chemoselectivity

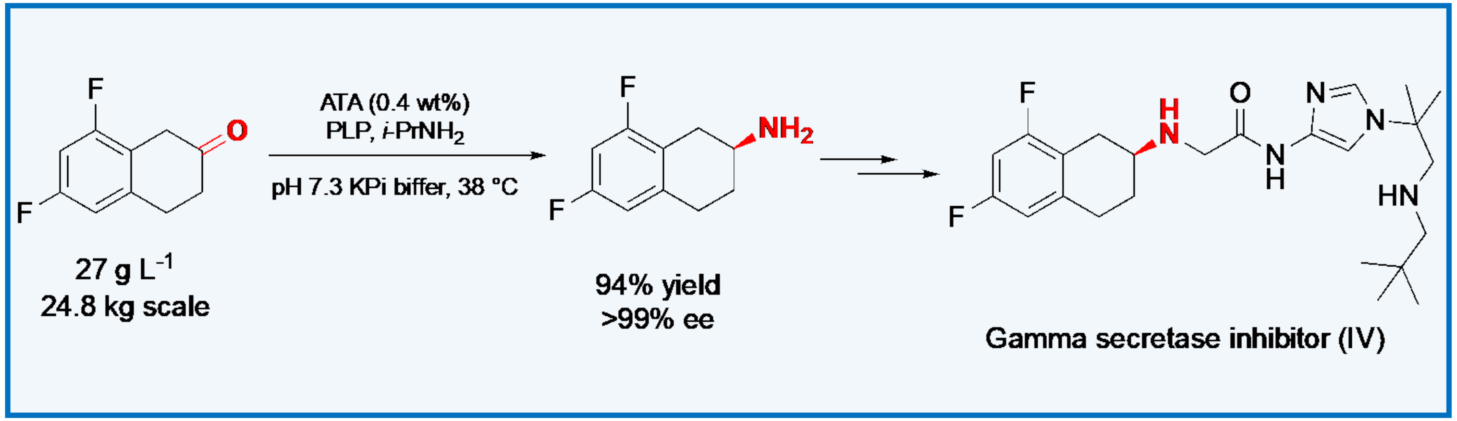
>95% *ee* (*R*)

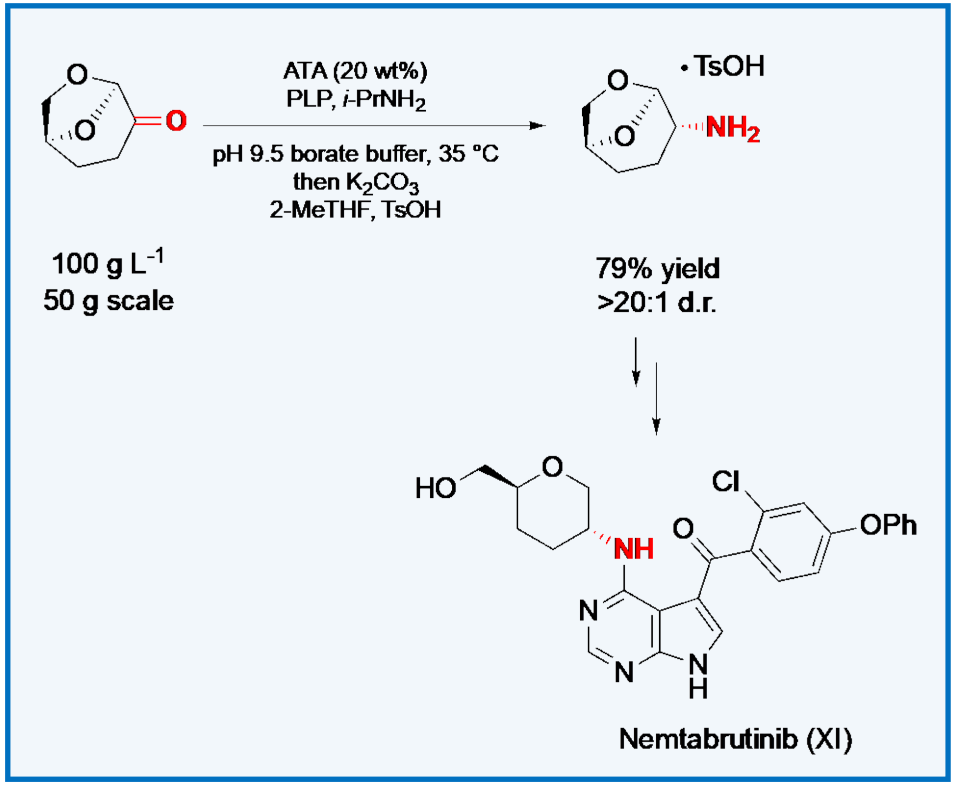


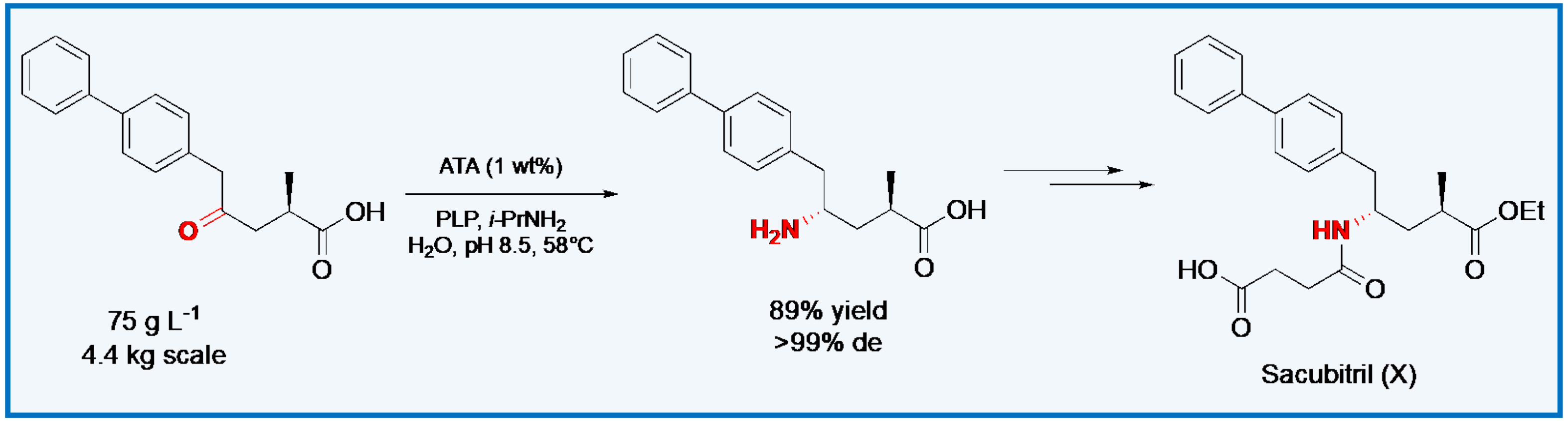
T. Knaus et al. *Org. Biomol. Chem.* **2017**, 15, 8313-8325

ω-TRANSAMINASES (ALSO CALLED AMINOTRANSFERASES) FOR APIS SYNTHESIS

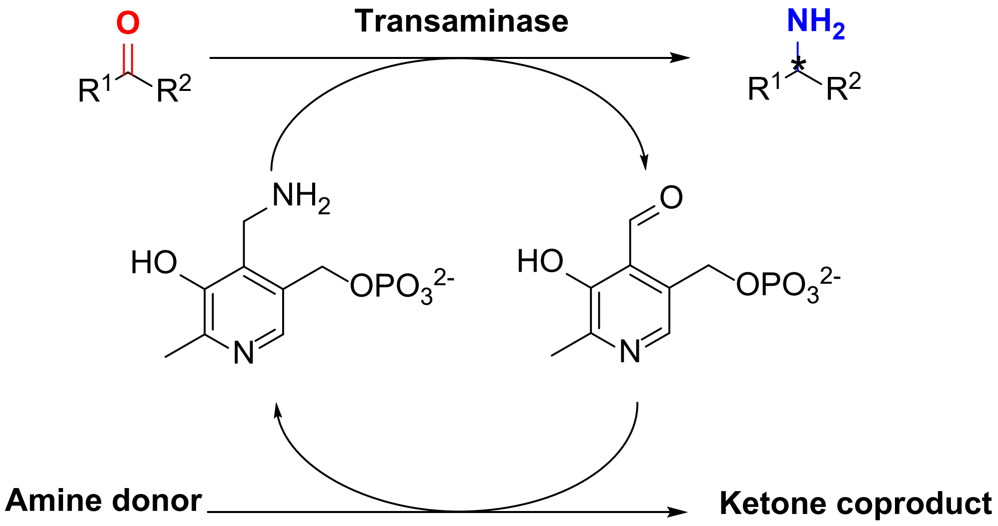
Examples for the use of aminotransferases (ATAs)



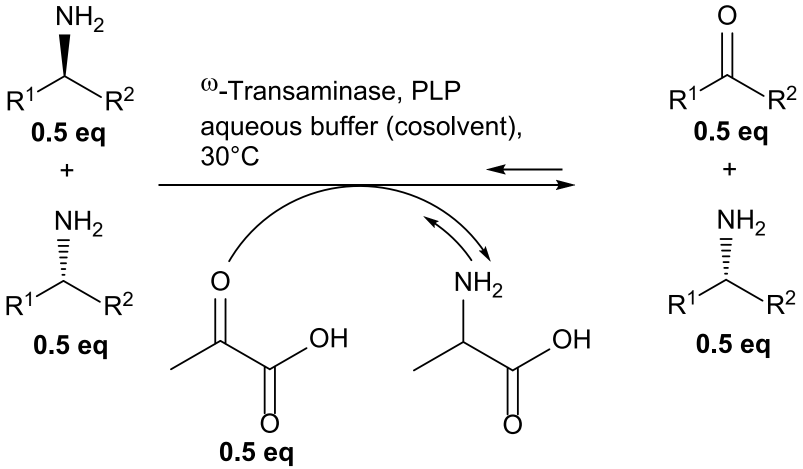




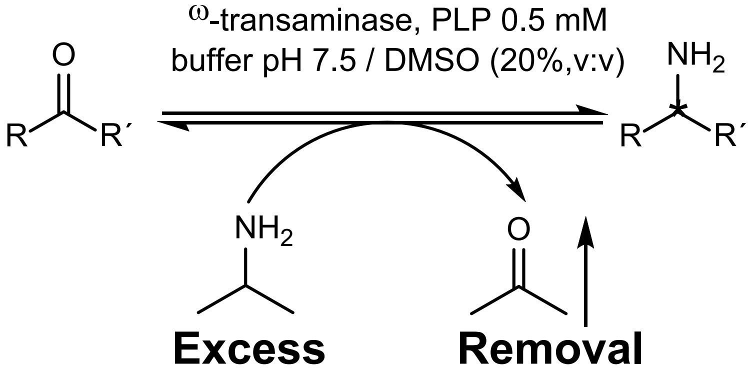
S. P. France, R. D. Lewis, C. A. Martinez, *JACS Au* **2023**, *3*, 715-735.



Kinetic resolution

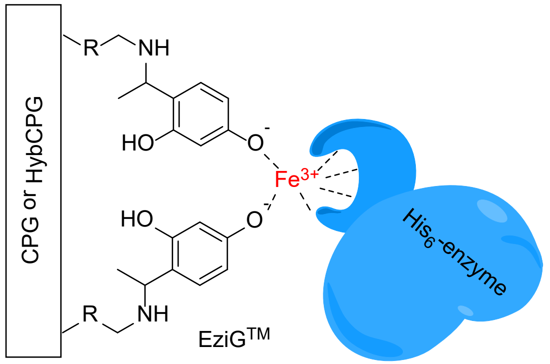


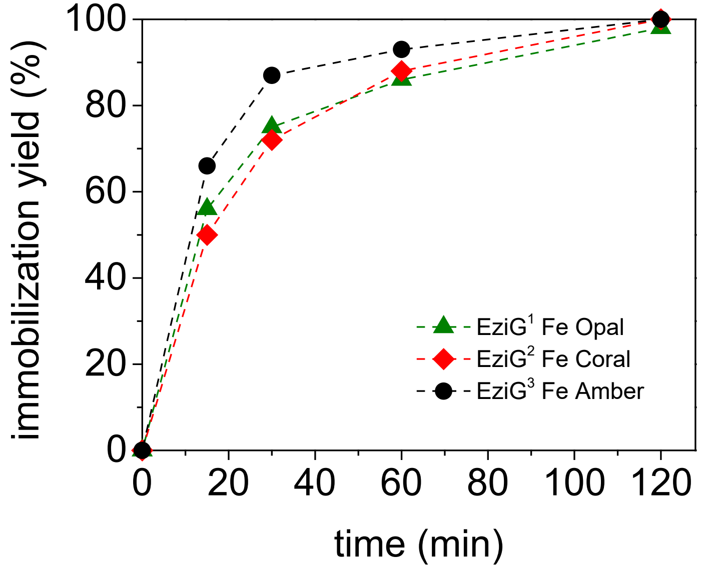
Asymmetric synthesis

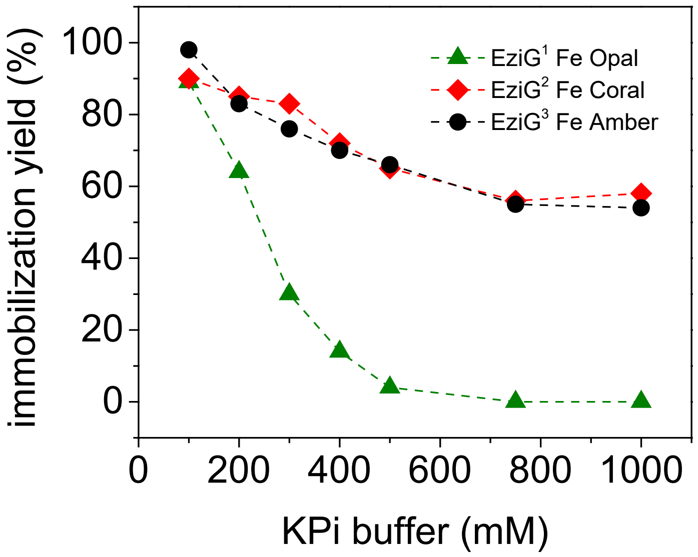


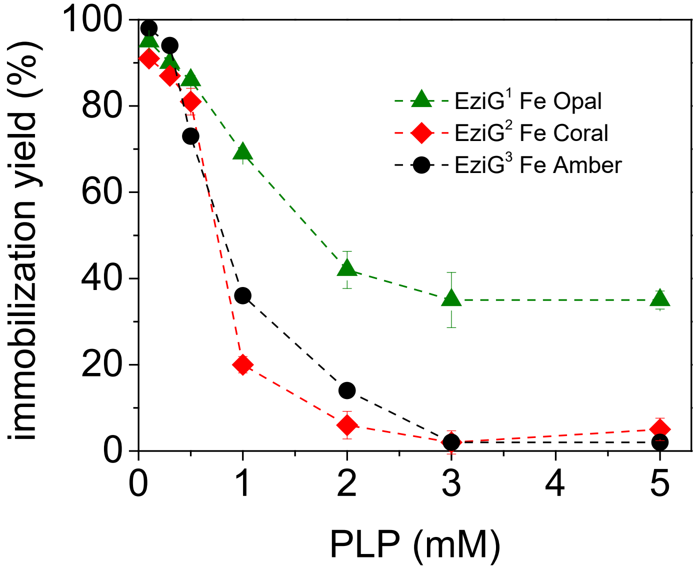
a) Kelly, S. A.; Pohle, S.; Wharry, S.; Mix, S.; Allen, C. C. R.; Moody, T. S.; Gilmore, B. F. *Chem. Rev.* **2018**, 118, 349-367; b) Guo, F.; Berglund, P. *Green Chem.* **2017**, 19, 333-36; c) Slabu, I.; Galman, J. L.; Lloyd, R. C.; Turner, N. J. *ACS Catal.* **2017**, 7, 8263-8284; d) Fuchs, M.; Farnberger, J. E.; Kroutil, W. *Eur. J. Org. Chem.* **2015**, 6965-6982; e) Mathew, S.; Yun, H. *ACS Catal.* **2012**, 2, 993-1001.

METAL ION AFFINITY IMMOBILIZATION OF TRANSAMINASES AND TESTS







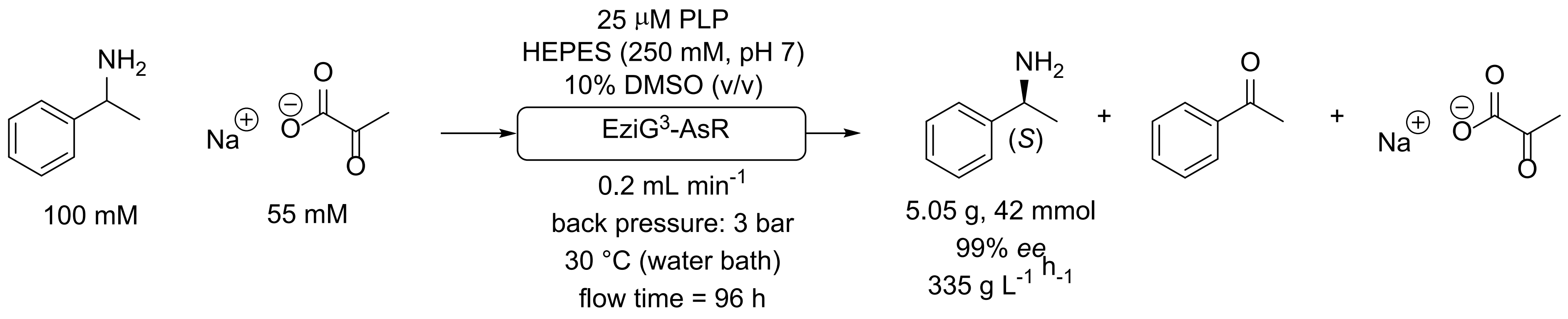


- Quick (2-3 h), quantitative and selective immobilization
- High enzyme loading (up to 25% w w^{-1})

- low KPi buffer ionic strength favours immobilisation yield
- low PLP concentrations is required to achieve high immobilization yield

W. Böhmer, T. Knaus, A. Volkov, T. K. Slot, N. Raveendran Shiju, K. Engelmark Cassimjee, F. G. Mutti *J. Biotechnol.* **2019**, *291*, 52-60.

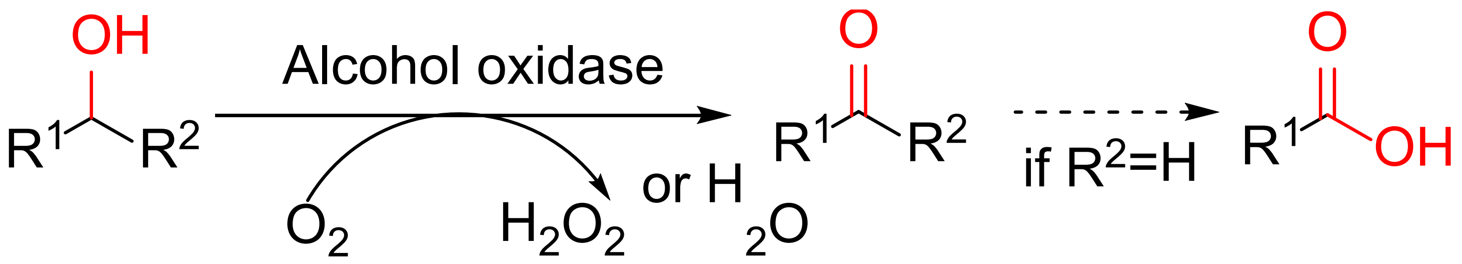
BIOCATALYSIS IN FLOW (AQUEOUS MEDIUM)

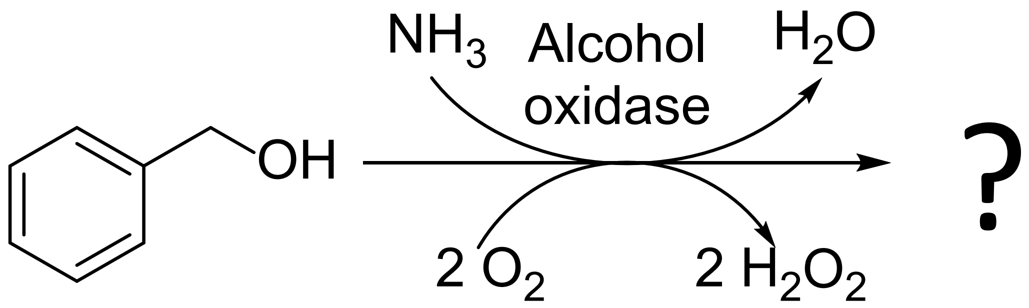


Packed-bed flow reactor (L= 50 mm; d= 2 mm): $V_{reactor}$ = 157 µL

- $[S]_0$ = 100 mM
- 10% w w^{-1} AsR-ω-TA on $EziG^3$ Fe-Amber
- Total TON 110000 (in 96 h operation)
- STY 0.34 kg L^{-1} h^{-1}

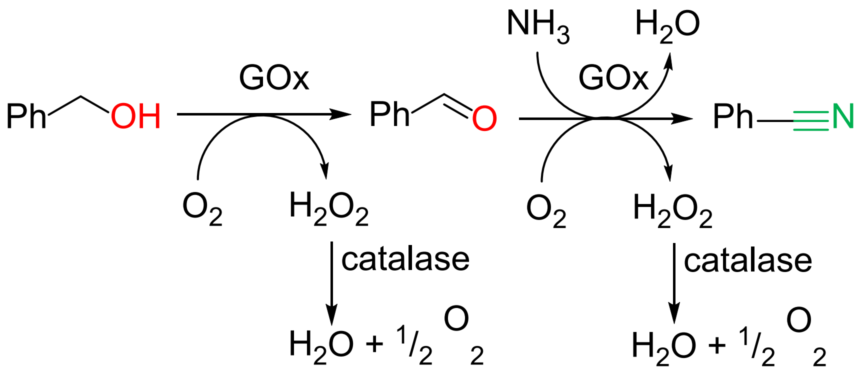
W. Böhmer, T. Knaus, A. Volkov, T. K. Slot, N. Raveendran Shiju, K. Engelmark Cassimjee, F. G. Mutti *J. Biotechnol.* **2019**, *291*, 52-60.

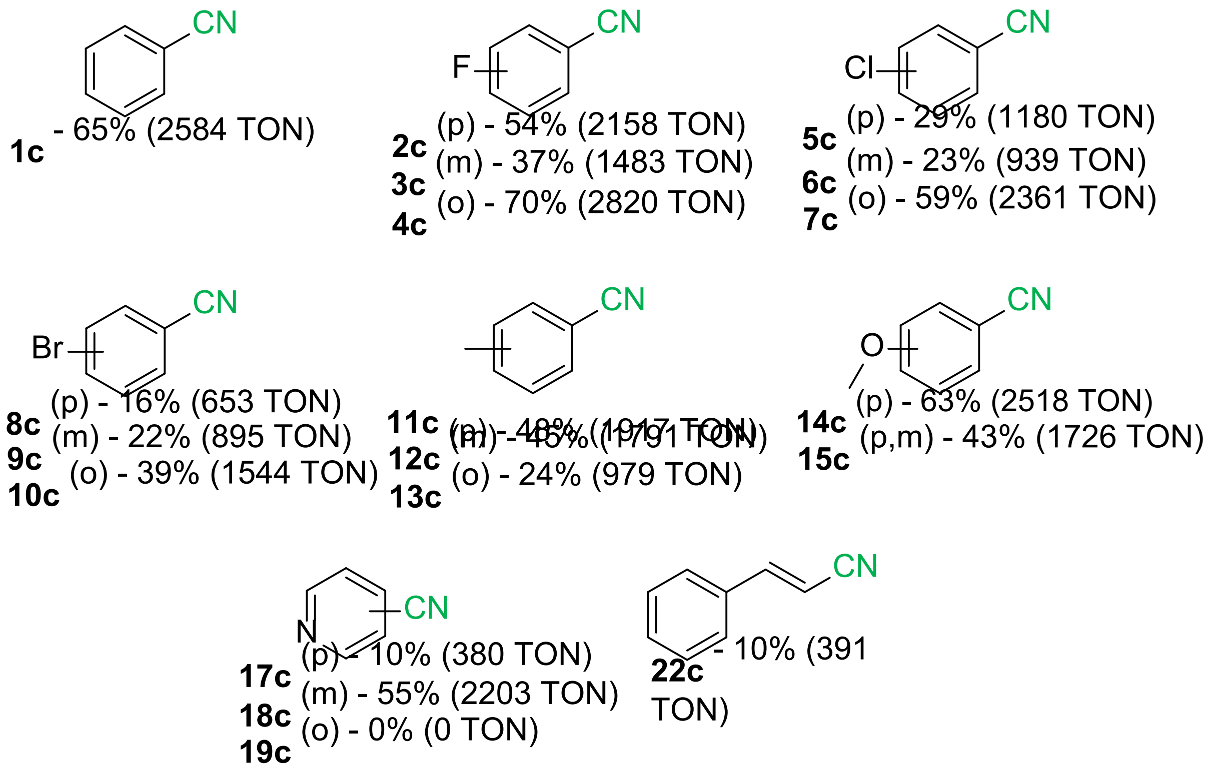

OH
R1
R2
Alcohol oxidase
O2
H2O2
or H2O
O
R1
R2
if R2=H
O
R1
OH


OH
NH3
Alcohol oxidase
H2O
2 O2
2 H2O2
?

NITRILE SYNTHESIS FROM ALCOHOL, AIR AND AMMONIA!

Optimization using cell extract of GOx





1) J. Vilím, T. Knaus, F. G. Mutti, *Angew. Chem. Int. Ed.* **2018**, 57, 14240-14244.
2) J. Vilím, T. Knaus, F. G. Mutti, WO 2020020844.

ACKNOWLEDGEMENTS

erc
European Research Council
Established by the European Commission

NWO
Netherlands Organisation for Scientific Research

国家留学基金管理委员会
CHINA SCHOLARSHIP COUNCIL

MARIE CURIE ACTIONS

RESEARCH PRIORITY AREA SUSTAINABLE CHEMISTRY

Holland Chemistry
Global Challenges, Breakthrough Solutions

HOLLAND RESEARCH SCHOOL OF MOLECULAR CHEMISTRY

HIMSBiocat
http://www.hims-biocat.eu/