ACS Green Chemistry Institute
Pharmaceutical Roundtable

Most commonly used biocatalytic transformations in descending order

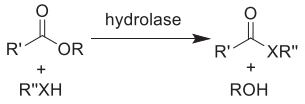
Name [generic; specific examples]
Key info

Scheme

Substrate scope:
RED = specific
GREEN = broad scope

Cofactor:
RED = multiple enzymes, or rarely used
YELLOW = commonly used, no second enzyme
GREEN = not required, no additional enzyme required

Hydrolase [lipases; esterases]
R, R', R" can be prochiral centers, often used for kinetic resolutions and desymmetrizations. Immobilization enables use of organic solvents.

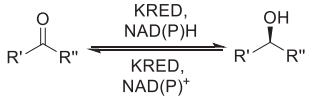


R"XH = water, alcohol, 1° and 2° amine

Penicillin G acylase [PGA]
Long historical use in antiobiotics. Narrow selectivity for R= phenacyl, phenoxyacyl, phenylglycinyl. Immobilization enables use of organic solvents.

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

Ketoreductase [KRED; carbonyl-reductase; alcohol dehydrogenase]
R- and *S*-selectivities available. Dynamic kinetic resolutions possible within R' and R" groups. Equilibrium usually favors alcohol product. Can be run in reverse oxidative direction.



substrate scope | cofactor

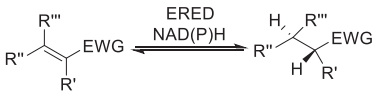
substrate scope

substrate scope

substrate scope | NAD(P)H

Transaminase [aminotransferase; ATA; TA; ω-TA]
R- and *S*-selectivities available. Dynamic kinetic resolutions possible within R' and R" groups. Drive equilibrium to desired amine through by-product removal.

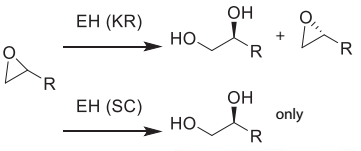
Enereductase [enoate reducatase, ERED]
Trans-reduction of the alkene. Selectivity can be engineered, steric crowding generally poorly tolerated. Drive equilibrium to desired product.



EWG : NO_2 > CHO > COR > CO_2R >CN

Nitrilase [NIT]
Irreversible conversion of nitrile to acid (enzymes that convert nitrile to amide are nitrile hydratases). Used in kinetic resolutions or chemoselective hydrolysis of one nitrile over another.

Epoxide hydrolase [EH]
Irreversible conversion of epoxide to diol. Mostly used for kinetic resolution (KR). Some EHs are stereoconvergent (SC), i.e. convert a racemic epoxide to single enantiomer diol. Different mechanistic classes exist.



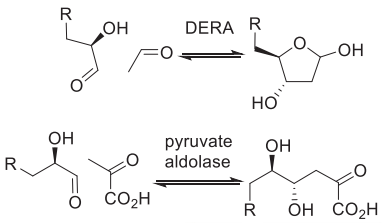
substrate scope | PLP

substrate scope | NAD(P)H

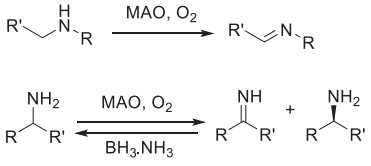
substrate scope

substrate scope

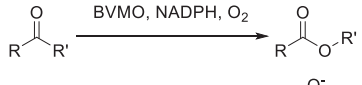
Aldolase
Several classes of aldolase: DERA, deoxyribose aldolase; others pyruvate and fructose aldolase.



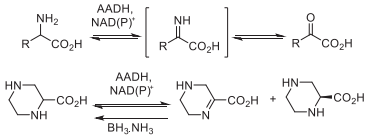
Monoamine oxidase [MAO]
Desymmetrization of pyrrolidines and trapping of imine. Primary amine oxidation and deracemization when coupled to compatible chemical reducing agent.



Baeyer-Villiger monooxygenase [BVMO; cyclohexane monooxygenase]
Asymmetric BV reaction, asymmetric sulfide oxidation to sulfoxide.



Amino acid dehydrogenase [AADH; LAADH; DAADH]
Most commonly used in the 'reverse' direction to form novel amino acids. *R*- and *S*-selective enzymes available. Deracemization of amines when coupled to compatible chemical reducing agent.



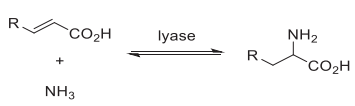
substrate scope

substrate scope

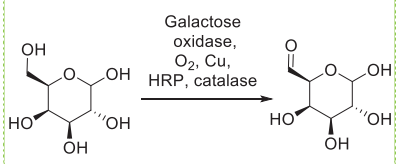
substrate scope | NAD(P)H

substrate scope | NAD(P)H

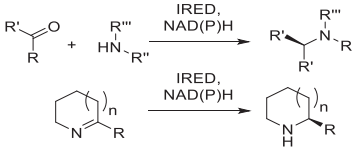
Ammonia lyase [amino acid ammonia lyase; PAL: phenylalanine ammonia lyase; TAL: tyrosine ammonia lyase]
Those are commonly used; others available. Used with very high ammonia concentrations to drive equilibrium towards formation of the amino acid.



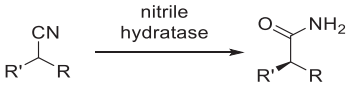
Alcohol oxidase [AO]
Many sub-types with different substrate selectivities; e.g., galactose oxidase (GO) acts on primary alcohols in polyols and benzylic alcohols. Kinetic resolutions possible. Oxygen mass transfer limited.



Imine reductase [IRED; reductive aminase: RedAm]: Asymmetric intermolecular reductive amination with IRED and RedAm. Some IRED only active on pre-formed imines



Nitrile hydratase
Irreversible conversion of nitrile to amide (enzymes that convert nitrile to acid are nitrilases). Kinetic or dynamic resolution possible with enolisable proton.



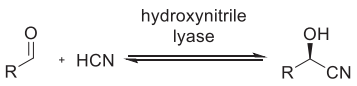
substrate scope | PLP

substrate scope | Cu, HRP, catalase

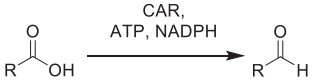
substrate scope | NAD(P)H

substrate scope

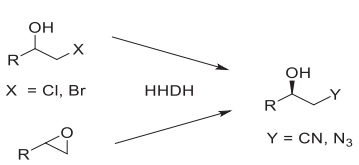
Hydroxynitrile lyase [HNL]
Catalyze the formation and hydrolysis of α-hydroxynitriles from aldehydes and a cyanide source. Used in a commercial manufacture of mandelic acid.



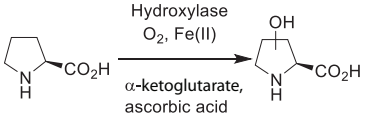
Carboxylic acid reductase [CAR]
Multidomain enzyme that uses ATP to transform the carboxylic acid to a thioester, and then reduces the thioester to the aldehyde using NADPH.



Halohydrin dehalogenase [HHDH]
Catalyze either the conversion of vicinal halohydrins to epoxides, or epoxide ring opening. Closely related to some epoxide hydrolases.



Amino acid hydroxylase
Non-heme Fe(II)- and α-ketoglutarate-dependent enzymes using O_2 as oxidant. Ascorbic acid generally required. Enzymes available for regio- and stereoselective hydroxylation of cyclic as well as acyclic amino acids.



substrate scope

substrate scope | NAD(P)H, ATP

substrate scope

substrate scope | Fe(II), ascorbate

For further information see the reagent guides at **reagents.acsgcipr.org**

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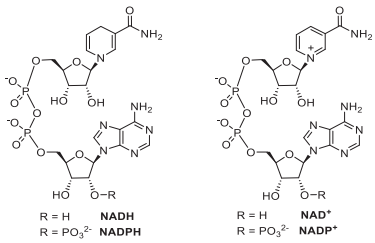
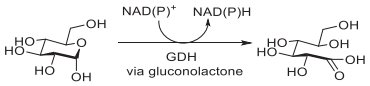
Nicotinamide cofactor recycling

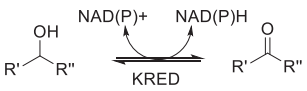
Nicotinamide Cofactor Recycling



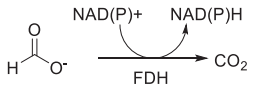
Glucose dehydrogenase [GDH]
Formation of gluconic acid drops reaction pH, and may require pH control. Highly active enzyme. Active on both NAD^+ and $NADP^+$



Ketoreductase [KRED; alcohol dehydrogenase]
Uses an alcohol, such as isopropanol, to reduce NAD(P)+ For ketone reductions the KRED often is dual purpose, reducing the desired substrate and oxidizing IPA. Reaction is reversible.

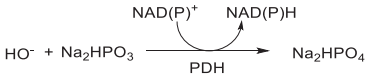


Formate dehydrogenase [FDH]
Irreversible conversion of formic acid salts to CO_2 generally less active than GDH. Often NAD selective.

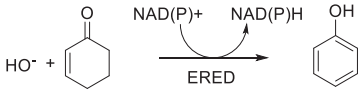


Commonly used --> rarely used

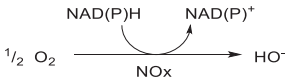
Phosphite dehydrogenase [PDH]
Generally NAD+ selective over NADP+, and typically less active than GDH.



Enereductase [enoate reductase, ERED]
Sacrifical substrate approach (similar to KRED + IPA). Use an unsaturated donor that aromatizes upon oxidation.



Oxidative Approaches
NAD(P)H oxidase [NOx].
Irreversible conversion of reduced co-factor to oxidized cofactor in presence of O_2. NADH selective enzymes are better characterized, though NADPH activity can be engineered.



Non-enzymatic methods

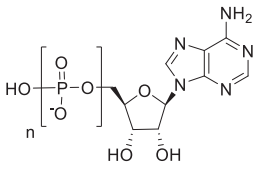
Electrochemical
Potentially the 'greenest' approach; though still in development.

Photochemical
Currently under development.

Non-abundant metal hydrogenation
Currently under development, but questionable long-term sustainability.

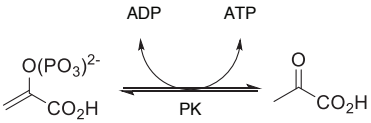
Adenosine triphosphate (ATP) recycling

Adenosine Triphophate (ATP) Recycling

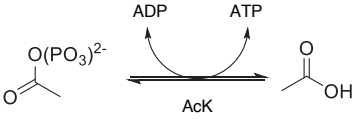


n = 0 **adenosine**
n = 1 **adenosine monophosphate (AMP)**
n = 2 **adenosine diphosphate (ADP)**
n = 3 **adenosine triphosphate (ATP)**

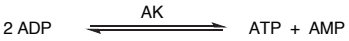
Phosphoenolpyruvate (PEP) kinase [PK]
PEP is expensive and primarily used in academic settings.



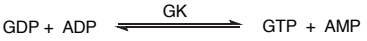
Acetate kinase [AcK]
Acetylphosphate is relatively easy to make, but hydrolytically unstable. Under-utilized in industry.



Adenylate kinase [AK]
Used in combination with another enzymes that convert AMP --> ADP (e.g., PPT).

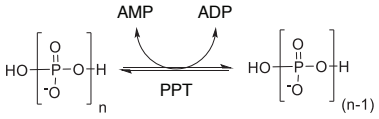


Guanylate kinase [GK]
Analagous to AK above (can also work with two molecules of GDP interconverting with GTP and GMP).



Commonly used --> rarely used

Polyphosphate transferase [PPT]
Polyphosphate is very cheap, and hydrolytically stable. Not all phosphate units are transferred. Under-utilized in industry.



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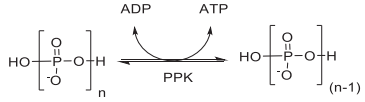
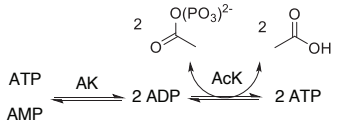


Combinations for recycling AMP to ATP
AK forms ADP, which is acted upon by AcK in presence of acetylphosphate to give ATP. PPK and polyphosphate can be used in place of AK and acetylpphosphate



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