ACS Green Chemistry Institute Pharmaceutical Roundtable

Most commonly used biocatalytical transformations

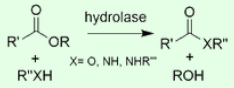
Name
[generic; specific examples]
Key info
Scheme

Substrate scope:
RED = specific
GREEN = broad scope

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

substrate scope

Hydrolase
[lipases, esterases, PGA]
R, R', R" can be asymm centers, often used for kinetic resolutions and desymmetrizations. When immobilized can tolerate organic solvents.

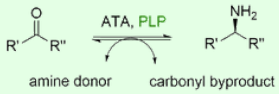


substrate scope

Ketoreductase
[KRED, carbonyl-reductase, alcohol dehydrogenase]
R- and *S*-selectivities available. Dynamic kinetic resolutions possible within R' and R" groups. Eqm usually favors alcohol product. Can run in oxidative direction. Additional cofactor recycling enzymes can be used.

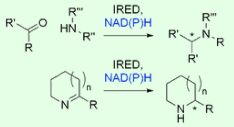
substrate scope

Transaminase
[aminotransferase, ATA, TA, *w*- TA]
R- and *S*-selectivities available. Dynamic kinetic resolutions possible. Eqm usually favors ketone, requires driving towards amine product.



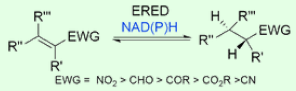
substrate scope

Imine reductase
[IRED, reductive aminase, amine dehydrogenase]
Asymmetric intermolecular reductive amination with IRED and RedAm. Some IRED only acitive on preformed imines



substrate scope

Enereductase
[enoate reducatase, ERED]
Trans reduction of the alkene. Selectivity can be engineered, steric crowding generally poorly tolerated. Eqm requires driving

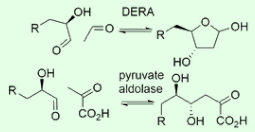


substrate scope

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.

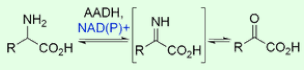
substrate scope

Aldolase
Several classes of aldolase, e.g.DERA (deoxyribose aldolase), others such as pyruvate and fructose aldolase also known.



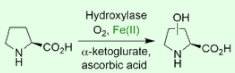
substrate scope

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 reductant.



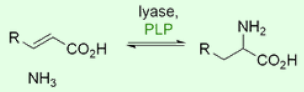
substrate scope

aKG dependent dioxygenase
[lipases, esterases, PGA]
Non-heme Fe(II)- and a-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. Non-amino acids can also be substrates.



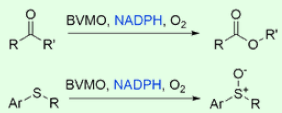
substrate scope

Ammonia lyase
[amino acid ammonia lyase]
PAL phenylalanine ammonia lyase, TAL tyrosine ammonia lyase most commonly used but others available. Used in the amino acid forming direction with very high ammonia concentrations to drive equilibrium.



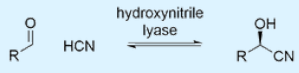
substrate scope

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



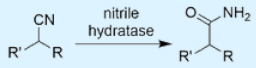
substrate scope

Hydroxynitrile lyase
[HNL]
Catalyze the formation and hydrolysis of a-hydroxy nitriles from/to aldehydes and cyanide. Used in a commercial approach to mandelic acid.



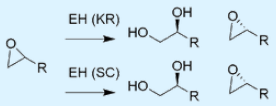
substrate scope

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



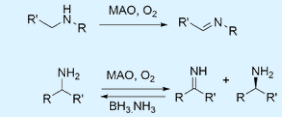
substrate scope

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



substrate scope

Monoamine oxidase
[MAO]
Desymmetrization of pyrrolidines, and trap of imine. Primary amine oxidation. Deracemization of amines when coupled to compatible chemical reductant.

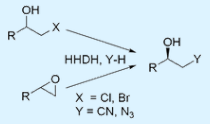


substrate scope

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

substrate scope

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



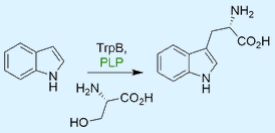
substrate scope

Unspecific peroxygenase
[UPO]
Fungal heme containing enzymes use hydrogen peroxide as oxidant and require no cofactors. They have varying oxidative capabilities including:

Hydroxylation, epoxidation, *N*- or *S*- oxidation, bromination, dealkylation

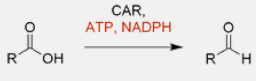
substrate scope

Tryptophan synthase
[TrpB]
Native reaction forms L-tryptophan using PLP cofactor. Many non-canonical amino acids have been produced with engineered variants.



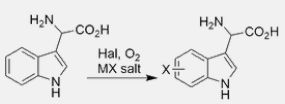
substrate scope

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 with NADPH.



substrate scope

Halogenase
Halogenation of aromatic rings. Halogenation takes place via a halogenated lysine residue. Regiochemistry can be controlled via directed evolution of the enzyme.



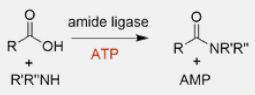
substrate scope

Cytochrome P450
[P450]
Heme containing enzyms using oxygen as oxidant.Requires electron transfer proteins either as part of the enzyme or added enzymes, often nicotinamide dependent. They have varying oxidative capabilities including:

Hydroxylation, desaturation, epoxidation, *N*- or *S*-oxidation, dealkylation

substrate scope

Amide ligase
[amide synthetase]
ATP dependent amide formation between acid and amine.



substrate scope

≥2 Peer reviewed examples of reactions scaled to ≥ 1 kg, or multiple double digit gram. Enzymes available at > 100 g scale

≥ 2 Peer reviewed examples of reactions scaled to ≥ 10 g

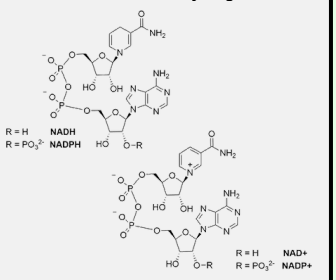
≥ 1 Peer reviewed example of reactions scaled to multi-mg

For references and to download, visit:
acsgcipr.org/tools/biocatalysis-guide/

ACS Green Chemistry Institute
Pharmaceutical Roundtable

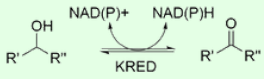
Nicotinamide cofactor recycling

Nicotinamide Cofactor Recycling

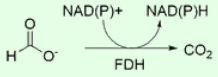


Glucose dehydrogenase
[GDH]
Gluconic acid formation drops reaction pH, and may required the use of a pH stat. Highly active enzyme. Active on both NAD+ and NADP+.

Ketoreductase
[KRED, alchol 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.

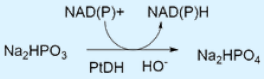


Formate dehydrogenase
[FDH]
Irreversible conversion of formic acid salts to CO2. Generally less active than GDH. Often NAD selective.



Most commonly used reductive nicotinamide regenerating systems

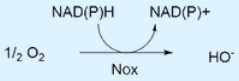
Phosphite dehydrogenase
[PDH]
Generally NAD+ selective over NADP+. Generally less active than GDH.



Enereductase
[enoate reducatase, ERED]
Sacrifical substrate approach (similar to KRED + IPA). Use unsaturated donor that can aromatize when oxidized.

For oxidative approaches

NAD(P)H oxidase
[Nox.]
Irreversible conversion of reduced co-factor to oxidized cofactor in presence of O2. NADH or NADPH activity available.



Non-enzymatic methods

Electrochemical
Potentially the 'greenest' approach, still in development.

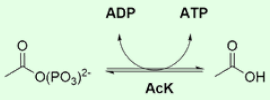
Photochemical
Still in development.

Non-abundant metal hydrogenation
Still in development, but questionable sustainability.

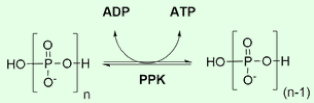
Less commonly used reductive nicotinamide regenerating systems

Adenosine triphosphate (ATP) recycling

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

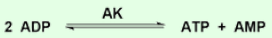


Polyphosphate kinase
[PPK]
Polyphosphate is very cheap, and hydrolytically stable. Not all phosphate units are transferred. Underused in industry.



Phosphoenolpyruvate kinase
[PK]
Phosphoenolpyruvate (PEP) is expensive, used mostly in academic settings

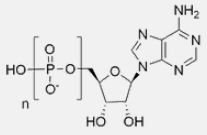
Adenylate kinase
[AK]
Used in combination with another enzymes that convert AMP --> ADP (e/g. PPT)



Guanylate kinase
GK]
Analagous to AK above (can work with 2 GDP interconverting with GTP and GMP)

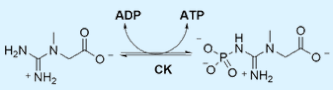
Most commonly used ATP regenerating systems

Adenosine Triphosphate (ATP) Recycling

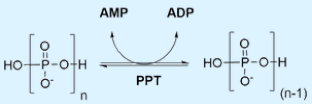


n = 0 adenosine
n = 1 adenosine monophosphate (AMP)
n = 2 adenosine diphosphate (ADP)
n = 3 adenosine triphosphate (ATP)

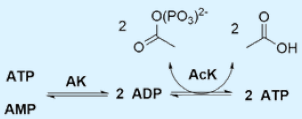
Creatine phosphate kinase
Stable creatine phosphate and thiophosphate is made chemically. Enzyme can transfer either phosphate or thiophosphate.



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



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 could be used in place of AK and acetylpphosphate.



Less commonly used ADP/ATP regenerating systems

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