

ACS GCIPR's Biocatalysis Guide: A Guide to the Most Common Transformations

Name

[generic; specific examples]

Scheme

Key info

Cofactor:

Bold = cofactor or sub-stoichiometric additive/enzyme
Bold + Italics = cofactor that requires a recycling system

Substrate scope:

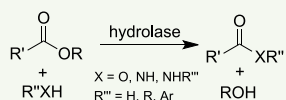
Broad to Medium to Limited

Most commonly used biocatalytical transformations

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

Hydrolase

[lipases, esterases, PGA]

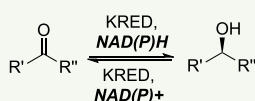


R', R'', R''' can contain asym centers, often used for kinetic resolutions and desymmetrizations. When immobilized can tolerate organic solvents.

BROAD SUBSTRATE SCOPE

Ketoreductase

[KRED, carbonyl-reductase, alcohol dehydrogenase]

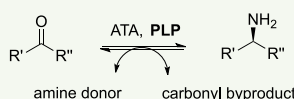


R- and S-selectivities available. Dynamic kinetic resolutions possible within R' and R'' groups. Eqm usually favors alcohol product. Can run in oxidative direction.

BROAD SUBSTRATE SCOPE

Transaminase

[aminotransferase, ATA, TA, w-TA]

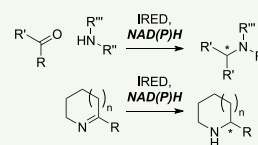


R- and S-selectivities available. Dynamic kinetic resolutions possible. Eqm usually favors ketone, requires driving towards amine product.

BROAD SUBSTRATE SCOPE

Iminoreductase

[IRED, reductive aminase, amine dehydrogenase]

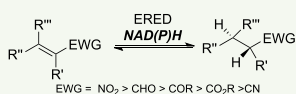


Asymmetric intermolecular reductive amination with IRED and RedAm. Some IRED only active on preformed imines.

MEDIUM SUBSTRATE SCOPE

Enereductase

[enoate reductase, ERED]



Trans reduction of the alkene. Selectivity can be engineered, steric crowding generally poorly tolerated. Eqm requires driving

LIMITED SUBSTRATE SCOPE

Nitrilase

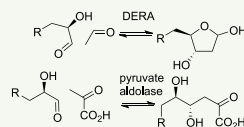
[NIT]



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.

MEDIUM SUBSTRATE SCOPE

Aldolase

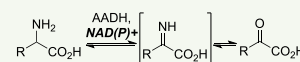


Several classes of aldolase, e.g. DERA (deoxyribose aldolase), others such as pyruvate and fructose aldolase also known.

LIMITED SUBSTRATE SCOPE

Amino acid dehydrogenase

[AADH, LAADH, DAADH]

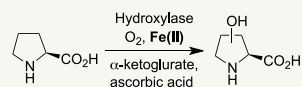


Most commonly used in the 'reverse' direction to form novel amino acids. R- and S- selective enzymes available. Deracemization of amines when coupled to compatible chemical reductant.

MEDIUM SUBSTRATE SCOPE

α-Ketoglutarate dependent dioxygenase

[lipases, esterases, PGA]

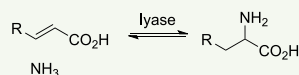


Non-heme Fe(II)- and α-ketoglutarate dependent enzymes using O2 as oxidant. Ascorbic acid generally required. Enzymes available for regio- and stereoselective hydroxylation of cyclic as well as acyclic amino acids. Non-amino acids can also be substrates.

LIMITED SUBSTRATE SCOPE

Ammonia lyase

[amino acid ammonia lyase]

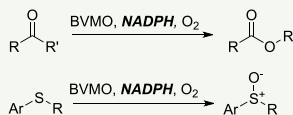


PAL phenylalanine ammonia lyase, TAL tyrosine ammonia lyase most commonly used but others available. Used in the amino acid forming direction with very high ammonia concentrations to drive equilibrium.

MEDIUM SUBSTRATE SCOPE

Baeyer-Villiger monooxygenase

[BVMO, cyclohexane monooxygenase]



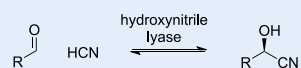
Asymmetric BV reaction, asymmetric sulfide oxidation to sulfoxide.

LIMITED SUBSTRATE SCOPE

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

Hydroxynitrile lyase

[HNL]

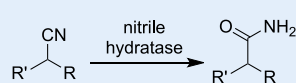


Catalyzes the formation and hydrolysis of α-hydroxy nitriles from/to aldehydes and cyanide. Used in a commercial approach to mandelic acid.

LIMITED SUBSTRATE SCOPE

Nitrile hydratase

[HNL]

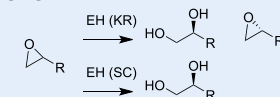


Irreversible conversion of nitrile to amide (enzymes that convert nitrile to acid are nitrilases). Kinetic or dynamic resolution possible with enolisable proton.

MEDIUM SUBSTRATE SCOPE

Epoxide hydrolase

[EH]

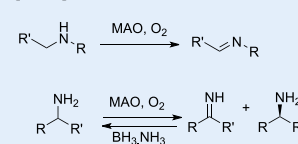


Irreversible conversion of epoxide to diol. Mostly used for kinetic resolution (KR). Some EHs are stereoconvergent (SC), ie convert a racemic epoxide to single enantiomer diol. Different mechanistic classes exist.

LIMITED SUBSTRATE SCOPE

Monoamine oxidase

[MAO]

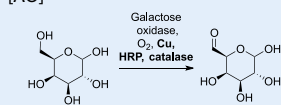


Desymmetrization of pyrrolidines, and trap of imine. Primary amine oxidation. Deracemization of amines when coupled to compatible chemical reductant.

MEDIUM SUBSTRATE SCOPE

Alcohol oxidase

[AO]

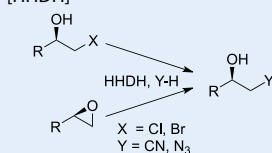


Many sub-types with different substrate selectivities e.g. galactose oxidase (GO) acts on primary alcohols in polyols and benzylic alcohols. Kinetic resolutions possible. Oxygen mass transfer limited.

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.

LIMITED SUBSTRATE SCOPE

Unspecific peroxygenase

[UPO]

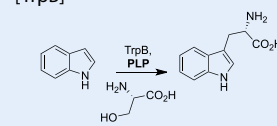
Fungal heme containing enzymes use hydrogen peroxide as oxidant and require no cofactors. They have varying oxidative capabilities including:

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

MEDIUM SUBSTRATE SCOPE

Tryptophan synthase

[TrpB]



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

LIMITED SUBSTRATE SCOPE

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

Carboxylic acid reductase

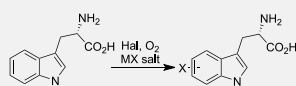
[CAR]



Multidomain enzyme that uses ATP to transform the carboxylic acid to a thioester, and then reduces the thioester to the aldehyde with NADPH.

LIMITED 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.

LIMITED SUBSTRATE SCOPE

Cytochrome P450

[P450]

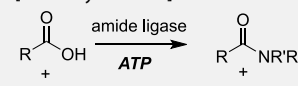
Heme containing enzymes using oxygen as oxidant. Requires electron transfer proteins either as part of the enzyme or added enzymes, often nicotinamide dependent. They have varying oxidative capabilities including:

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

LIMITED SUBSTRATE SCOPE

Amide ligase

[amide synthetase]

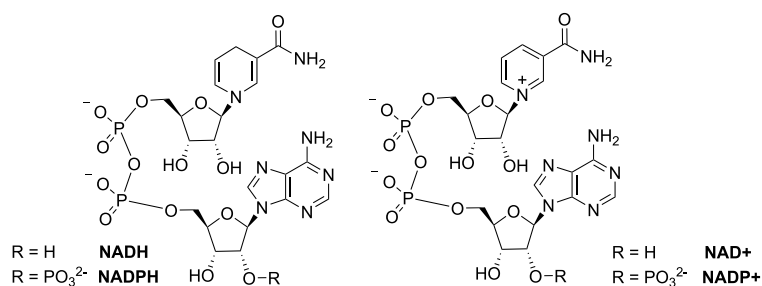


ATP dependent amide formation between acid and amine.

LIMITED SUBSTRATE SCOPE

For further information see the reagent guides at reagents.acsgcpi.org

Nicotinamide cofactor recycling



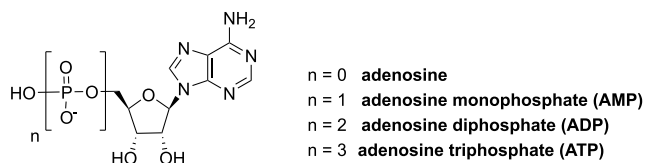
Most commonly used reductive nicotinamide regenerating systems

Glucose dehydrogenase [GDH] Gluconic acid formation drops reaction pH, and may require the use of a pH stat. 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 CO₂. Generally less active than GDH. Often NAD selective.
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Less commonly used reductive nicotinamide regenerating systems

Phosphite dehydrogenase [PDH] Generally NAD⁺ selective over NADP⁺. Generally less active than GDH.	Enoreductase [enoate reductase, ERED] Sacrificial substrate approach (similar to KRED + IPA). Use unsaturated donor that can aromatize when oxidized.	NAD(P)H oxidase [Nox] For oxidative approaches Irreversible conversion of reduced co-factor to oxidized cofactor in presence of O₂. NADH or NADPH activity available. Sacrificial substrate approach (similar to KRED + IPA). Use unsaturated donor that can aromatize when oxidized.	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.
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Adenosine triphosphate (ATP) recycling



Most commonly used ATP regenerating systems

Acetate kinase
[AcK]

Acetylphosphate is relatively easy to make, but hydrolytically unstable. Underused in industry.

Polyphosphate kinase
[PPK]

Type III PPK can convert AMP to ATP, other classes convert AMP to ADP. 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).

Less commonly used ADP/ATP regenerating systems

Creatine 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 acetylphosphate.