

ROBOX

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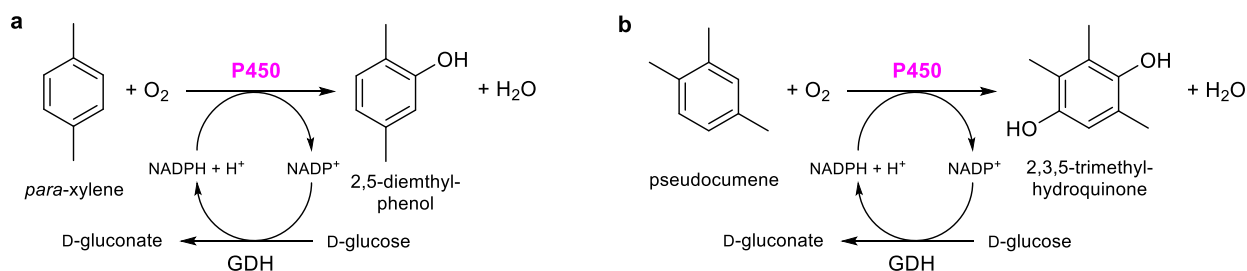


ROBOX Technology Information

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ROBOX Demonstration 1 Fact Sheet P450 Aromatics

- Demonstration: Aromatic hydroxylation by P450
- Reaction scheme:

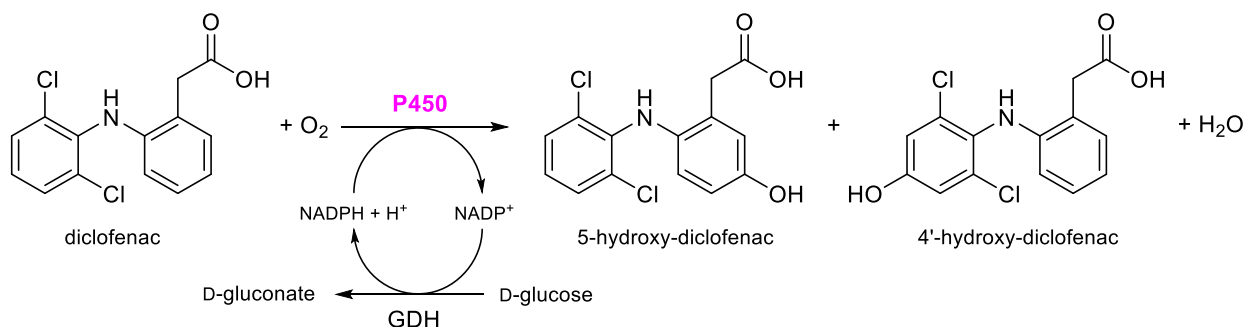


- Demonstrating project partner: InnoSyn B.V.
- Demonstration enzymes:
 - P450 *para*-xylene to 2,5-dimethylphenol: P450-BM3 variant M2 (3-fold variant)
 - P450 pseudocumene to trimethyl-hydroquinone: P450-BM3 variant B11 (4-fold variant)
 - GDH: GDH-02 from thermophilic bacterial species
- Producer demonstration enzymes: InnoSyn B.V. (co-expression of P450 and GDH in one high cell-density *Escherichia coli* fermentation)
- Demonstration scale: 1 l reaction volume in lab-scale biooxidation reactor
- Oxidant: pressurised pure O₂
- Product produced: 0.3 – 3.3 g hydroxylated aromatics
- co-factor required yes/no:
 - yes: NADPH
- Selectivity (enantio-selectivity: E-value or e.e. value; regio- and chemoselectivity):
 - Regio-selectivity: >70% aromatic to <30% benzylic hydroxylation
 - Enantioselectivity: not applicable
- Application fields:
 - Intermediates for fine chemicals (vitamins and nutritional products)
- Process metrics:

Process metric	<i>para</i> -xylene	pseudocumene
Reaction conversion [%]	29	22
Reaction yield [%]	22	2.2
Product concentration [g/l]	3.3	0.29
Volumetric Productivity / STY [g l ⁻¹ h ⁻¹]	0.15	0.012
Biocatalyst loading [g _{cell wet weight} /l]	43	58
Biocatalyst yield [g _{product} /g _{cell wet weight}]	0.08	0.005

ROBOX Demonstration 2 Fact Sheet P450 API metabolites

- Demonstration: Diclofenac metabolite production by P450
- Reaction scheme:

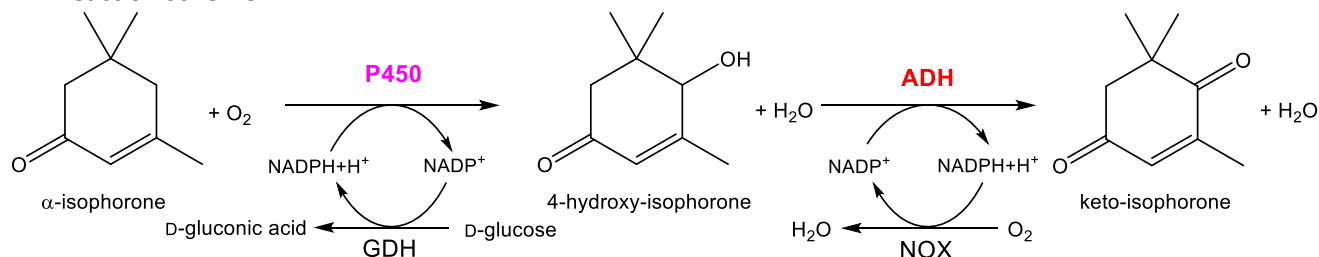


- Demonstrating project partner: InnoSyn B.V.
- Demonstration enzymes:
 - P450: P450-BM3 variant 22C02 (5-fold variant, P450-002)
 - GDH: GDH-02 from thermophilic bacterial species
- Producer demonstration enzymes: InnoSyn B.V. (co-expression in one high cell-density *Escherichia coli* fermentation)
- Demonstration scale: 2 x 100 l reaction volume in 200 l reactor
- Oxidant: O₂ from pressurised air
- Product produced: 660 g hydroxylated diclofenac metabolites
- co-factor required yes/no:
 - yes: NADPH
- Selectivity (enantio-selectivity: E-value or e.e. value; regio- and chemoselectivity):
 - Regio-selectivity: 70% 4'-hydroxy- to 30% 5-hydroxy-diclofenac
 - Enantioselectivity: not applicable (aromatic hydroxylation)
- Application fields:
 - Active pharmaceutical ingredient (API) metabolite for toxicity- and regulatory studies
 - API modification for increased solubility and/or biological activity
- Process metrics:

Process metric	1 l scale	100 l scale
Reaction conversion [%]	93	99
Reaction yield [%]	93	99
Product concentration [g/l]	3.5	3.0
Volumetric Productivity / STY [g l ⁻¹ h ⁻¹]	0.4	0.34
Biocatalyst loading [g _{cell wet weight} /l]	63	28
Biocatalyst yield [g _{product} /g _{cell wet weight}]	0.07	0.12

ROBOX Demonstration 3 Fact Sheet P450 Alkenes

- Demonstration: Alkene hydroxylation by P450
- Reaction scheme:

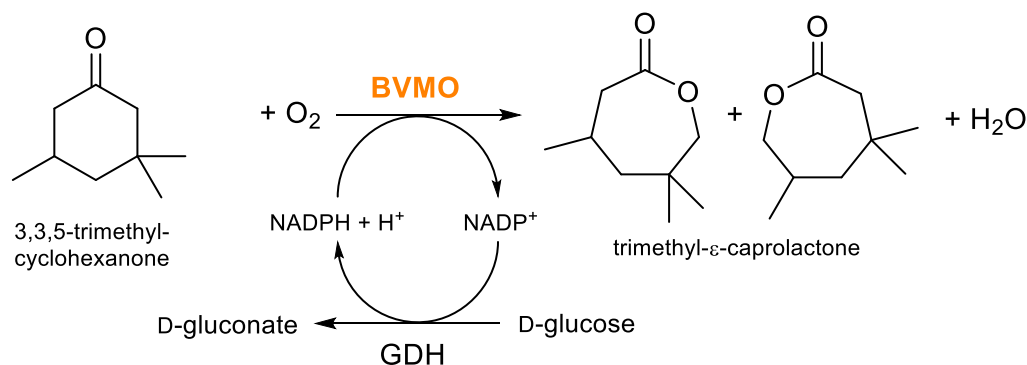


- Demonstrating project partner: InnoSyn B.V.
- Demonstration enzymes:
 - P450: Cytochrome P450-BM3 hydroxylase variant M2 (3-fold variant, P450-003)
 - GDH: Glucose Dehydrogenase from thermophilic *Bacillus* species (GDH-01)
 - ADH: Alcohol Dehydrogenase ADH-R variant E-3
 - NOX: NAD(P)H Oxidase variant (2-fold variant, NOX-01)
- Producer demonstration enzymes:
 - ADH-R variant E-3: evovx (by high cell-density *Escherichia coli* fermentation)
 - P450-003, GDH-01 and NOX-01: InnoSyn B.V. (by high cell-density *E. coli* fermentations)
- Demonstration scale: 100 l reaction volume in 200 l reactor
- Oxidant: pressurised pure O_2
- Product produced: 0.5 kg 4-hydroxy-isophorone
- co-factor required yes/no:
 - yes: NADPH
- Selectivity (enantio-selectivity: E-value or e.e. value; regio- and chemoselectivity):
 - Regio-selectivity: >85% aromatic 4-OH- to 10% 3-OH-methyl-isophorone to 5% epoxide
 - Enantioselectivity: not relevant/determined
- Application fields:
 - Intermediate for fine chemicals (vitamins and nutritional products)
- Process metrics (P450 step only):

Process metric	1 l scale	100 l scale
Reaction conversion [%]	58	78
Reaction yield [%]	55	61
Product concentration [g/l]	11	2.3
Volumetric Productivity / STY [g l ⁻¹ h ⁻¹]	1.5	0.51
Biocatalyst loading [g _{cell wet weight} /l]	87	50
Biocatalyst yield [g _{product} /g _{cell wet weight}]	0.15	0.05

ROBOX Demonstration 4 Fact Sheet BVMO trimethyl-cyclohexanone

- Demonstration: trimethyl-cyclohexanone BVMO to trimethyl- ϵ -caprolactones (TMCL)
- Reaction scheme:

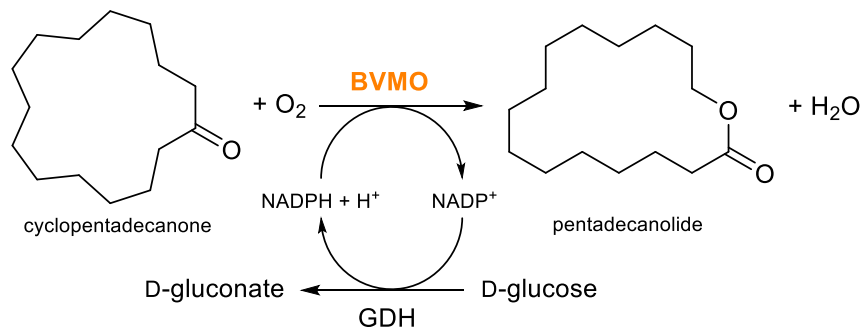


- Demonstrating project partner: InnoSyn B.V.
- Demonstration enzymes:
 - BVMO: *Thermocrisum municipale* Cyclohexanone Monooxygenase (TmCHMO)
 - GDH: Glucose Dehydrogenase from thermophilic *Bacillus* species (GDH-01)
- Producer demonstration enzymes: InnoSyn B.V.
- Demonstration scale: 100 l reaction volume in 200 l reactor
- Oxidant: pressurised pure O₂
- Product produced: 2.6 kg trimethyl- ϵ -caprolactones (isolated, 85% chemical purity)
- co-factor required yes/no:
 - yes: NADPH
- Selectivity (enantio-selectivity: E-value or e.e. value; regio- and chemoselectivity):
 - Regioselectivity: 60% β,β,δ -TMCL to 40% β,δ,δ -TMCL
 - Enantioselectivity: not determined
- Application fields:
 - Monomer for specialty polymers
- Process metrics:

Process metric	1 l scale	100 l scale
Reaction conversion [%]	97	85
Reaction yield [%]	97	85
Product concentration [g/l]	36	24
Volumetric Productivity / STY [g l ⁻¹ h ⁻¹]	6.0	2.7
Biocatalyst loading TmCHMO [g _{cell wet weight} /l]	46	41
Biocatalyst loading GDH-01 [g _{cell wet weight} /l]	1.7	1.0
Total biocatalyst loading [g _{cell wet weight} /l]	48	42
Biocatalyst yield TmCHMO [g _{product} /g _{cell wet weight}]	0.8	0.6
Biocatalyst yield GDH-01 [g _{product} /g _{cell wet weight}]	21	24
Total biocatalyst yield [g _{product} /g _{cell wet weight}]	0.8	0.6

ROBOX Demonstration 5 Fact Sheet BVMO cyclopentadecanone

- Demonstration: cyclopentadecanone BVMO to pentadecanolide
- Reaction scheme:

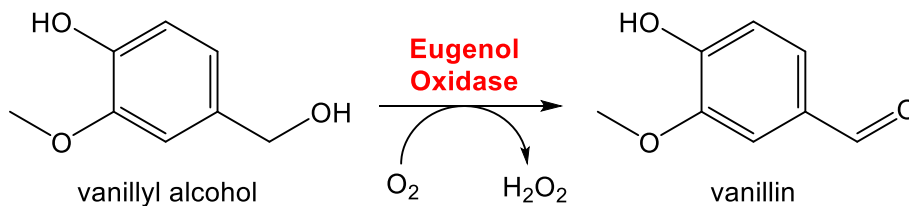


- Demonstrating project partner: InnoSyn B.V.
- Demonstration enzymes:
 - BVMO: *Rhodococcus ruber* Cyclododecanone Monooxygenase (RrCDMO)
 - GDH: Glucose Dehydrogenase from thermophilic *Bacillus* species (GDH-01)
- Producer demonstration enzymes: InnoSyn B.V.
- Demonstration scale: 100 l reaction volume in 200 l reactor
- Oxidant: pressurised pure O₂
- Product produced: 4.4 kg pentadecanolide (isolated, 86% chemical purity)
- co-factor required yes/no:
 - yes: NADPH
- Selectivity (enantio-selectivity: E-value or e.e. value; regio- and chemoselectivity):
 - Not applicable (symmetric, not pro-chiral substrate)
- Application fields:
 - Pentadecanolide: Monomer for specialty polymers
 - Pentadecanolide analogues:
 - Flavour & Fragrance: musk lactones
 - Monomers for specialty polymers
- Process metrics:

Process metric	1 l scale	100 l scale
Reaction conversion [%]	96	98
Reaction yield [%]	96	92
Product concentration [g/l]	57	40
Volumetric Productivity / STY [g l ⁻¹ h ⁻¹]	8.2	4.0
Biocatalyst loading RrCDMO [g _{cell wet weight} /l]	33	56
Biocatalyst loading GDH-01 [g _{cell wet weight} /l]	0.7	0.7
Total biocatalyst loading [g _{cell wet weight} /l]	34	57
Biocatalyst yield RrCDMO [g _{product} /g _{cell wet weight}]	2.1	0.8
Biocatalyst yield GDH-01 [g _{product} /g _{cell wet weight}]	99	56
Total biocatalyst yield [g _{product} /g _{cell wet weight}]	2.0	0.8

ROBOX Demonstration 6 Fact Sheet AOX vanillin

- Demonstration: vanillyl alcohol AOX to vanillin
- Reaction scheme:

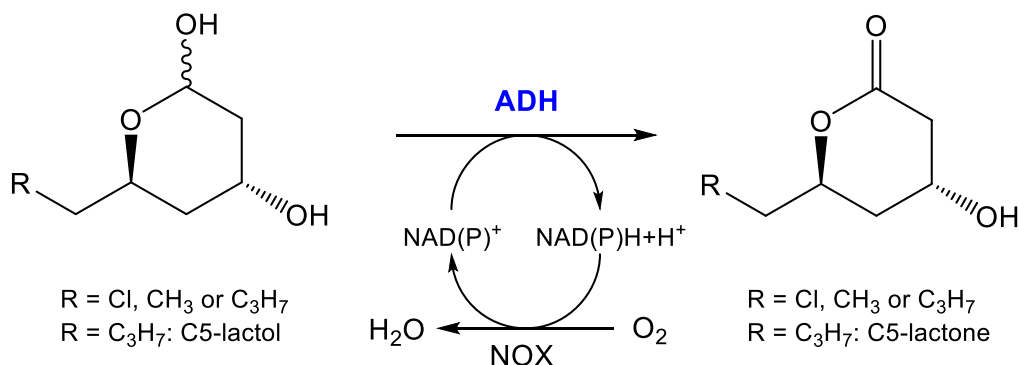


- Demonstrating project partner: InnoSyn B.V.
- Demonstration enzymes:
 - AOX: *Rhodococcus jostii* Eugenol Oxidase (EUGO)
 - H_2O_2 quenching: chemical
- Producer demonstration enzymes: InnoSyn B.V.
- Demonstration scale: 100 l reaction volume in 200 l reactor
- Oxidant: pressurised pure O_2
- Product produced: 3.8 kg vanillin
- co-factor required yes/no:
 - no
- Selectivity (enantio-selectivity: E-value or e.e. value; regio- and chemoselectivity):
 - Not applicable
- Application fields:
 - (natural) vanillin from (natural) vanillyl alcohol for F&F applications
- Process metrics:

Process metric	0.5 l scale A	0.5 l scale B	100 l scale
Reaction conversion [%]	99	97	95
Reaction yield [%]	97	94	82
Product concentration [g/l]	45	43	38
Volumetric Productivity / STY [$\text{g l}^{-1} \text{h}^{-1}$]	18	6.0	4.2
Biocatalyst loading [$\text{g}_{\text{cell wet weight}}/\text{l}$]	33	16	16
Biocatalyst yield [$\text{g}_{\text{product}}/\text{g}_{\text{cell wet weight}}$]	1.4	2.7	2.4

ROBOX Demonstration 7a Fact Sheet ADH lactol

- Demonstration: C5-lactol to C5-lactone oxidation by ADH
- Reaction scheme:

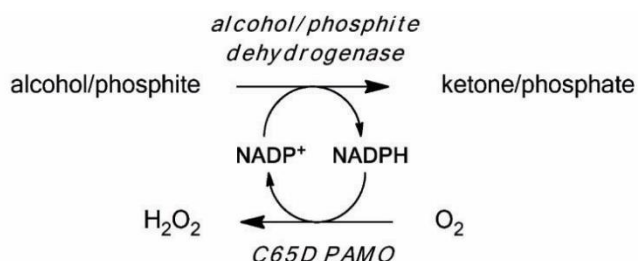


- Demonstrating project partner: InnoSyn B.V.
- Demonstration enzymes:
 - ADH: ADH-99 produced in high cell-density *Escherichia coli* fermentation by c-LEcta
 - NOX: NOX-01 produced in high cell-density *Escherichia coli* fermentation by InnoSyn
- Demonstration scale: 75 l reaction volume in 200 l reactor
- Oxidant: pressurised pure O₂
- Product produced: 3.6 kg C5-lactone
- co-factor required yes/no:
 - yes: NADPH
- Selectivity (enantio-selectivity: E-value or e.e. value; regio- and chemoselectivity):
 - Chemo-/regio-selectivity: >99% (no ketone detected)
 - Enantioselectivity: not applicable
- Application fields:
 - C5-lactol: precursor to Flavour & Fragrance lactones
 - Chlorolactone analogue: intermediate of Statin-processes (pharma chemical)
- Process metrics:

Process metric	0.5 l scale	75 l scale
Reaction conversion [%]	99	96
Reaction yield [%]	99	95
Product concentration [g/l]	49	47
Volumetric Productivity / STY [g l ⁻¹ h ⁻¹]	12.3	6.3
Biocatalyst loading ADH-99 [g _{cell wet weight} /l]	12	12
Biocatalyst loading NOX-01 [g _{cell wet weight} /l]	17	24
Total biocatalyst loading [g _{cell wet weight} /l]	29	36
Biocatalyst yield ADH-99 [g _{product} /g _{cell wet weight}]	4.1	2.9
Biocatalyst yield NOX-01 [g _{product} /g _{cell wet weight}]	3.9	2.0
Total biocatalyst yield [g _{product} /g _{cell wet weight}]	1.7	1.3

Enzyme: PAMO C65D (NADPH-Oxidase)
Source organism: *Thermobifida fusca*
Inventor: Molecular Enzymology Group, University of Groningen
Producer: Molecular Enzymology Group, University of Groningen
Activity (Steady-state kinetic parameters for NADPH oxidation): k_{cat} 5.0 s^{-1} , K_M $3.5 \text{ }\mu\text{M}$, k_{cat} / K_M $1.4 \text{ s}^{-1} \text{ }\mu\text{M}^{-1}$
Substrate: NADPH in combination with dehydrogenase and appropriate dehydrogenase substrate (e.g. alcohol / phosphite dehydrogenase)

Reaction scheme:



Cofactor required

Yes for the oxidation of NADPH

Selectivity (enantio-selectivity: E-value or E.E. value; region- and chemoselectivity)

Not applicable

Volumetric productivity / space-time yields (gram per liter and hour) achieved with this enzyme

Not applicable

Biocatalyst yields (kg product per kg biocatalyst (cell wet weight equivalents)) achievable with this enzyme

Not applicable

Enzyme activity and stability profile

pH range: not applicable
 pH optimum (for activity): 7.5
 pH optimum (for stability): 7.5
 Temperature range: not applicable
 Temperature optimum (for biocat application): 30 - 40°C

Solvent tolerance

Water-miscible solvents: e.g. DMSO and methanol up to 10% (v/v)
 Water non-miscible solvents: not applicable

Substrate scope and (activity on) model substrates

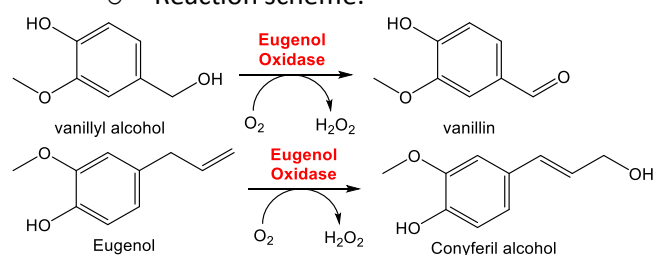
Tested with phosphite and PTDH as regenerating enzyme
 Tested with 2-butanol, cyclohexanol and *rac*-1-phenylethanol in combination with ADH from *Thermoanaerobacter brockii* or ADH evo-1.1.270 as regenerating enzyme

Biocatalytic system cost (fermentation volume and time)

Not applicable

ROBOX Enzymes Fact Sheet EUGO (RUG & InnoSyn)

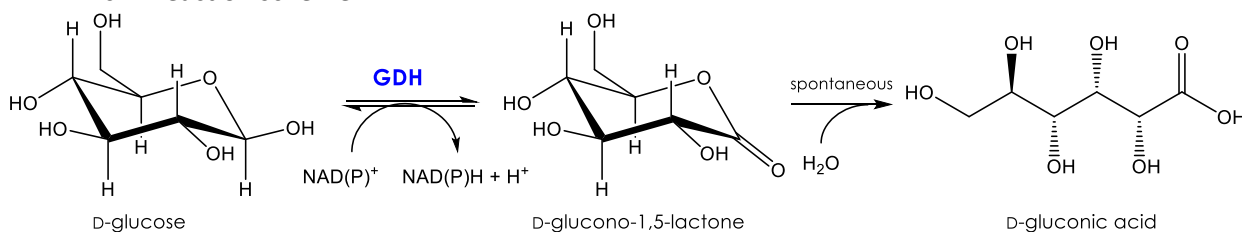
- Enzyme: Eugenol Oxidase EUGO
- Source organism: *Rhodococcus jostii* RHA-1
- Inventor: GECCO, University of Groningen
- Producer: InnoSyn B.V.
- Activity:
 - 0.95 U/mg total cell-free protein on vanillyl alcohol (at pH 7.5 and 30°C)
- Substrates:
 - Vanillyl alcohol or eugenol with O₂
 - Also other *para*-substituted phenols are accepted as substrate, such as zingerone and raspberry ketone
 - Reaction scheme:



- Cofactor required yes/no:
 - no
- Selectivity (enantio-selectivity: E-value or e.e. value; regio- and chemoselectivity):
 - No overoxidation to carboxylic acids observed
 - Capable of hydroxylating 4-allylphenols
 - Capable of kinetic resolution of 4-(1-hydroxyethyl)-2-methoxyphenol (E=35)
- Volumetric productivity / space-time yields (gram per liter and hour) achieved with this enzyme:
 - 0.5 g l⁻¹ h⁻¹ (in EUGO oxidation of vanillyl alcohol to vanillin on 100 l scale, 3.8 kg product produced)
- biocatalyst yields (kg product per kg biocatalyst (cell wet weight equivalents)) achieved with this enzyme:
 - 0.3 kg/kg (in EUGO oxidation of vanillyl alcohol to vanillin)
- Enzyme activity & stability profile
 - pH range: 4.0 – 10.0
 - pH optimum: 8.5 – 9.5
 - temperature range: 4 – 40°C
 - temperature optimum (for biocat application): 20 – 30°C
- Solvent tolerance:
 - Water-miscible solvents: DMSO, short chain alcohols and ketones up to 20% (v/v)
- Substrate scope and (activity on) model substrate(s):
 - phenolic alcohols and alkenes
- Biocatalytic system cost (fermentation volume and time):
 - high-cell density *Escherichia coli* fermentation (proven in 104 h fermentations at up to 15 l scale)
 - fermentation yields of > 43 kU EUGO activity per liter fermentation volume
 - fermentation yields of > 625 kU EUGO activity per kg cell dry weight

ROBOX Enzymes Fact Sheet GDH-01 (InnoSyn)

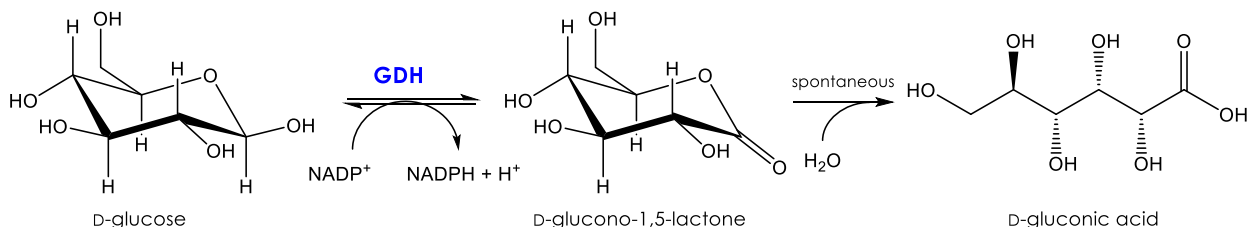
- Enzyme: Glucose Dehydrogenase GDH-01
- Source organism: thermophilic *Bacillus* species
- Inventor: InnoSyn B.V.
- Producer: InnoSyn B.V.
- Activity:
 - 100 U/mg total cell-free protein on NAD⁺ (at pH 8.0 and 30°C)
 - 70 U/mg total cell-free protein on NADP⁺ (at pH 8.0 and 30°C)
- Substrate:
 - Glucose with NAD⁺ or NADP⁺
 - Reaction scheme:



- co-factor required yes/no:
 - for NADH and NADPH regeneration
- Selectivity (enantio-selectivity: E-value or e.e. value; regio- and chemoselectivity):
 - Not applicable (dependent on the RedOx enzyme it is used with)
- Volumetric productivity / space-time yields (gram per liter and hour) achieved with this enzyme:
 - 4.0 g l⁻¹ h⁻¹ (in co-factor regeneration of BVMO reaction of cyclopentadecanone to pentadecanolide on 100 l scale, 4.4 kg isolated product)
 - 2.7 g l⁻¹ h⁻¹ (in co-factor regeneration of BVMO reaction of 3,3,5-trimethyl-cyclohexanone to 3,3,5-trimethyl-hexanolides on 100 L scale, 2.6 kg isolated product)
- biocatalyst yields (kg product per kg biocatalyst (cell wet weight equivalents)) achieved with this enzyme:
 - 60 kg/kg (in BVMO reaction of cyclopentadecanone to pentadecanolide)
- Enzyme activity & stability profile
 - pH range: 5.0 – 10.0
 - pH optimum (for activity): 7.0 – 10.0
 - pH optimum (for stability): 5.0 – 7.0
 - temperature range: 4 – 50°C
 - temperature optimum (for biocat application): 25 – 35°C
- Solvent tolerance:
 - Water-miscible solvents: e.g. methanol up to 20% (v/v)
 - Water non-miscible solvents: e.g. heptane and toluene at 50% (v/v)
- Substrate scope and (activity on) model substrate(s):
 - Not applicable here (co-factor regeneration enzyme)
- biocatalytic system cost (fermentation volume and time):
 - high-cell density *Escherichia coli* fermentation (proven in 100 h fermentations at up to 15 l scale)
 - fermentation yields of > 3.5 MU GDH activity per liter fermentation volume
 - fermentation yields of > 55 MU GDH activity per kg cell dry weight

ROBOX Enzymes Fact Sheet GDH-02 (InnoSyn)

- Enzyme: Glucose Dehydrogenase GDH-02
- Source organism: thermophilic Archaeon
- Inventor: InnoSyn B.V.
- Producer: InnoSyn B.V.
- Activity:
 - 20 U/mg total cell-free protein on NADP⁺ (at pH 8.0 and 30°C)
- Substrate:
 - Glucose with NADP⁺
 - Reaction scheme:



- co-factor required yes/no:
 - for NADPH regeneration
- Selectivity (enantio-selectivity: E-value or e.e. value; regio- and chemoselectivity):
 - dependent on the RedOx enzyme it is used with
 - strictly NADP⁺ specific
- Volumetric productivity / space-time yields (gram per liter and hour) achieved with this enzyme:
 - 6.0 g l⁻¹ h⁻¹ (in co-factor regeneration of P450 hydroxylation of α-isophorone to 4-hydroxy-isophorone on 100 l scale, 1.0 kg isolated product)
 - 0.7 g l⁻¹ h⁻¹ (in co-factor regeneration of P450 hydroxylation of on 100 l scale, 0.7 kg isolated product)
- biocatalyst yields (kg product per kg biocatalyst (cell wet weight equivalents)) achieved with this enzyme:
 - 0.1 kg/kg (limited by co-expressed P450, in hydroxylations of diclofenac, α-isophorone and *para*-xylene)
- Enzyme activity & stability profile
 - pH range: 6.0 – 9.0
 - pH optimum (for stability): 7.0 – 8.0
 - temperature range: 20 – 70°C
 - temperature optimum (for biocat application): 25 – 40°C
- Solvent tolerance:
 - Water-miscible solvents: e.g. methanol, ethanol or acetone at ≤ 50% (v/v)
 - Water non-miscible solvents: e.g. heptane and toluene at ≤ 50% (v/v)
- Substrate scope and (activity on) model substrate(s):
 - strictly NADP⁺ specific (co-factor regeneration enzyme)
- biocatalytic system cost (fermentation volume and time):
 - high-cell density *Escherichia coli* fermentation (proven in 102 h fermentations at up to 15 l scale)
 - fermentation yields of > 1.0 MU GDH activity per liter fermentation volume
 - fermentation yields of > 11 MU GDH activity per kg cell dry weight

ROBOX Enzymes Fact Sheet NOX-01 (InnoSyn)

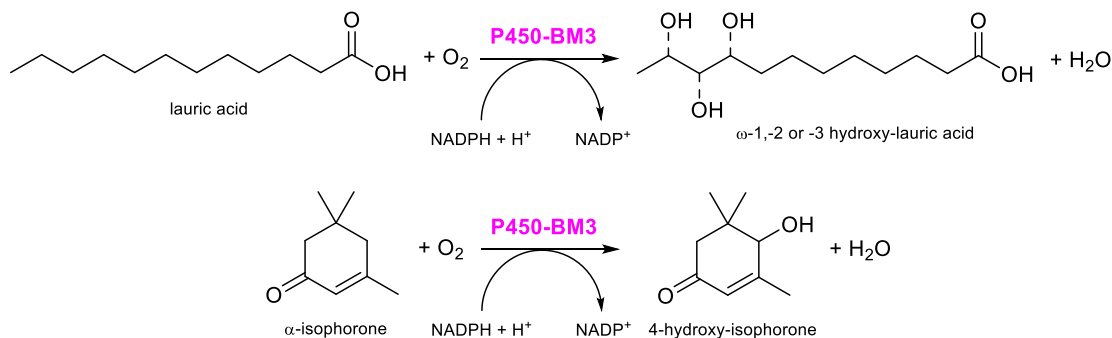
- Enzyme: NAD(P)H Oxidase NOX-01 (double mutant)
- Source organism: *Streptococcus mutans*
- Inventor: InnoSyn B.V.
- Producer: InnoSyn B.V.
- Activity:
 - 15 U/mg total cell-free protein on NADH (at pH 7.5 and 20°C)
 - 10 U/mg total cell-free protein on NADPH (at pH 7.5 and 20°C)
- Substrates:
 - NADH or NADPH with O₂
 - Reaction scheme:



- co-factor required yes/no:
 - for NAD⁺ and NADP⁺ regeneration
- Selectivity (enantio-selectivity: E-value or e.e. value; regio- and chemoselectivity):
 - Not applicable (dependent on the RedOx enzyme it is used with)
- Volumetric productivity / space-time yields (gram per liter and hour) achieved with this enzyme:
 - 6.3 g l⁻¹ h⁻¹ (in co-factor regeneration of ADH oxidation of C5-lactol to C5-lactone on 75 l scale, 3.6 kg product produced)
 - 2.4 g l⁻¹ h⁻¹ (in co-factor regeneration of ADH reaction of 4-hydroxy-isophorone to keto-isophorone on 30 mL scale, 0.22 g product produced)
- biocatalyst yields (kg product per kg biocatalyst (cell wet weight equivalents)) achieved with this enzyme:
 - 1.3 kg/kg (in ADH oxidation of C5-lactol to C5-lactone)
 - 1.3 kg/kg (in ADH oxidation of 4-hydroxy-isophorone to keto-isophorone)
- Enzyme activity & stability profile
 - pH range: 5.0 – 9.0
 - pH optimum: 6.0 – 8.0
 - temperature range: 4 – 37°C
 - temperature optimum (for biocat application): 20 – 30°C
- Solvent tolerance:
 - Water-miscible solvents: e.g. methanol and NMP up to 10% (v/v)
 - Water non-miscible solvents: e.g. 2-octanone and 2-ethyl-hexanol at 50% (v/v)
- Substrate scope and (activity on) model substrate(s):
 - Not applicable here (co-factor regeneration enzyme)
- biocatalytic system cost (fermentation volume and time):
 - high-cell density *Escherichia coli* fermentation (proven in 126 h fermentations at up to 15 l scale)
 - fermentation yields of > 440 kU NOX activity per liter fermentation volume
 - fermentation yields of >11 MU NOX activity per kg cell dry weight

ROBOX Enzymes Fact Sheet P450-001 (InnoSyn)

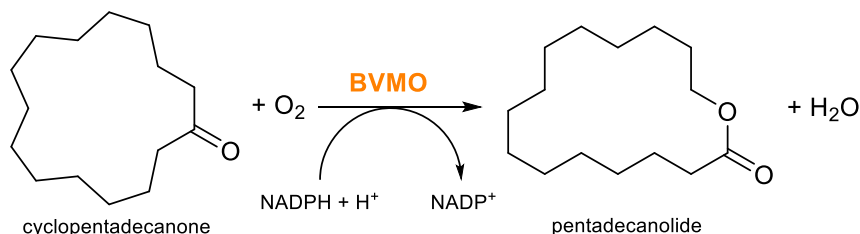
- Enzyme: Cytochrome P450 hydroxylase CYP102A1 (P450-BM3)
- Source organism: *Bacillus megaterium* ATCC 14581
- Innovator: InnoSyn B.V.
- Producer: InnoSyn B.V.
- Activity:
 - 0.26 U/mg total cell-free protein on lauric acid (at pH 7.5 and 25°C)
- Substrates:
 - Lauric acid with NADPH and O₂
 - Reaction scheme:



- co-factor required yes/no:
 - NADPH
- Selectivity (enantio-selectivity: E-value or e.e. value; regio- and chemoselectivity):
 - Depending on the substrate employed (96% regio-selectivity for 4-position of α-isophorone)
- Volumetric productivity / space-time yields (gram per liter and hour) achieved with this enzyme:
 - 1.5 g l⁻¹ h⁻¹ (in hydroxylation of α-isophorone to 4-hydroxy-isophorone on 100 l scale, 1.1 kg isolated product)
- biocatalyst yields (kg product per kg biocatalyst (cell wet weight equivalents)) achieved with this enzyme:
 - 0.1 kg/kg (in hydroxylation of α-isophorone to 4-hydroxy-isophorone)
- Enzyme activity & stability profile
 - pH range: 6.0 – 9.0
 - pH optimum: 7.0 – 8.0
 - temperature range: 4 – 40°C
 - temperature optimum (for biocat application): 15 – 30°C
- Solvent tolerance:
 - Water-miscible solvents: methanol, acetonitrile, THF and DMSO up to 5% (v/v)
- Substrate scope and (activity on) model substrate(s):
 - Fatty acids, (hetero-) aromatics, (hetero-)alkanes and alkenes (optionally cyclic)
 - Hydroxylations, epoxidations and dealkylations (depending on the substrate)
- biocatalytic system cost (fermentation volume and time):
 - batch *Escherichia coli* fermentations (proven in 22 h fermentations at 1000 l scale)
 - fermentation yields of > 1.5 μmol P450-BM3 per liter fermentation volume
 - fermentation yields of > 380 μmol P450-BM3 per kg cell dry weight
 - high-cell density *Escherichia coli* fermentations (proven in 54 h fermentations at 15 l scale)
 - fermentation yields of > 10 μmol P450-BM3 per liter fermentation volume
 - fermentation yields of > 270 μmol P450-BM3 per kg cell dry weight

ROBOX Enzymes Fact Sheet RrCDMO (InnoSyn)

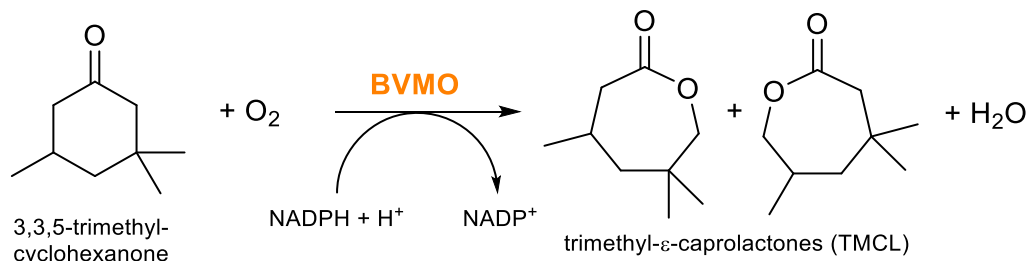
- Enzyme: Cyclododecanone Monooxygenase RrCDMO
- Source organism: *Rhodococcus ruber*
- Innovator: InnoSyn B.V.
- Producer: InnoSyn B.V.
- Activity:
 - 0.33 U/mg total cell-free protein on cyclopentadecanone (at pH 9.0 and 30°C)
- Substrates:
 - Cyclopentadecanone with NADPH and O₂
 - Reaction scheme:



- co-factor required yes/no:
 - NADPH
- Selectivity (enantio-selectivity: E-value or e.e. value; regio- and chemoselectivity):
 - Not yet investigated
- Volumetric productivity / space-time yields (gram per liter and hour) achieved with this enzyme:
 - 4.0 g l⁻¹ h⁻¹ (in BVMO reaction of cyclopentadecanone to pentadecanolide on 100 l scale, 4.4 kg isolated product)
- biocatalyst yields (kg product per kg biocatalyst (cell wet weight equivalents)) achieved with this enzyme:
 - 0.8 kg/kg (in BVMO reaction of cyclopentadecanone to pentadecanolide)
- Enzyme activity & stability profile
 - pH range: 7.0 – 10.0
 - pH optimum: 8.0 – 8.5
 - temperature range: 4 – 40°C
 - temperature optimum (for biocat application): 20 – 30°C
- Solvent tolerance:
 - Water-miscible solvents: methanol and DMSO up to 25% (v/v)
 - Water non-miscible solvents: cyclohexane and toluene at 50% (v/v)
- Substrate scope and (activity on) model substrate(s):
 - Macrocyclic ketones and medium sized cyclic ketones with large substituents
- biocatalytic system cost (fermentation volume and time):
 - high-cell density *Escherichia coli* fermentation (proven in 102 h fermentations at up to 15 l scale)
 - fermentation yields of > 8.2 kU RrCDMO activity per liter fermentation volume
 - fermentation yields of > 110 kU RrCDMO activity per kg cell dry weight

ROBOX Enzymes Fact Sheet TmCHMO (RUG & InnoSyn)

- Enzyme: Cyclohexanone Monooxygenase TmCHMO
- Source organism: *Thermocristpum municipale*
- Inventor: GECCO, University of Groningen
- Producer: InnoSyn B.V.
- Activity:
 - 0.12 U/mg total cell-free protein on cyclohexanone (at pH 9.0 and 30°C)
- Substrates:
 - cyclohexanone with NADPH and O₂
 - Reaction scheme:



- Cofactor required yes/no:
 - NADPH
- Selectivity (enantio-selectivity: E-value or e.e. value; regio- and chemoselectivity):
 - Regioselectivity: 60% β,β,δ -TMCL to 40% β,δ,δ -TMCL
 - Displays a similar broad substrate acceptance compared with CHMO from *Acinetobacter*, including (cyclic)ketones, sulphides, aldehydes.
- Volumetric productivity / space-time yields (gram per liter and hour) achieved with this enzyme:
 - 2.7 g l⁻¹ h⁻¹ (in BVMO reaction of trimethyl-cyclohexanone to trimethyl- ϵ -caprolactones on 100 l scale, 2.6 kg isolated product)
- biocatalyst yields (kg product per kg biocatalyst (cell wet weight equivalents)) achieved with this enzyme:
 - 0.6 kg/kg (in BVMO reaction of trimethyl-cyclohexanone to trimethyl- ϵ -caprolactones)
- Enzyme activity & stability profile
 - pH range: 7.0 – 10.0
 - pH optimum: 7.0 – 9.0
 - temperature range: 4 – 40°C
 - temperature optimum (for biocat application): 20 – 30°C
- Solvent tolerance:
 - Water-miscible solvents: e.g. methanol and ethanol up to 30% (v/v)
 - Water non-miscible solvents: e.g. toluene at 30% (v/v)
- Substrate scope and (activity on) model substrate(s):
 - Mainly explored for medium sized cyclic and linear ketones
- Biocatalytic system cost (fermentation volume and time):
 - high-cell density *Escherichia coli* fermentation (proven in 101 h fermentations at up to 15 l scale)
 - fermentation yields of > 6.1 kU TmCHMO activity per liter fermentation volume
 - fermentation yields of > 69 kU TmCHMO activity per kg cell dry weight